MEDIATORS OF THE TEMPERATURE CHANGES EVOKED BY INTRACEREBRAL INJECTIONS OF 5-HT IN THE CAT

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BY

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To Judy and Beth

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I. INTRODUCTION

There is evidence that 5-hydroxytryptamine (5-HT) is a synaptic transmitter in the mammalian central nervous system (CNS). The monoamine and the enzymes involved in its synthesis and metabolism are known to be present in the brain (Amin et al., 1954; Vogt, 1954; Bogdanski et al., 1957; Kuntzman et al., 1961; Guroff and Udenfriend, 1962; Gal et al., 1963). Subcellular fractionation studies have shown that 5-HT is localized in the synaptosomal fraction of mammalian brain homogenates (Whittaker, 1965; DeRobertis, 1967; Neal and Iversen, 1969) and investigations employing the fluorescent histochemical technique have permitted the visual identification of serotonergic cell bodies and axon terminals. It is now well established that the cell bodies containing 5-HT are located predominantly within the midline raphé nuclei of the pons and mesencephalon (Dahlstrom and Fuxe, 1965; Fuxe, 1965) and that the axons of these cells ascend mainly in the medial forebrain bundle to terminate in diencephalic and telencephalic structures (Fuxe et al., 1968). Furthermore, the suprachiasmatic nucleus receives the highest concentration of serotonin-containing nerve terminals (Fuxe et al., 1968).

Electrical stimulation of the midbrain raphé has provided evidence of a neuronal release of 5-HT from brain regions innervated by serotonergic neurons (Aghajanian et al., 1967; Randic and Padjen, 1968; Holman and Vogt, 1970).

Moreover, Bloom et al. (1972) demonstrated that electrical stimulation of the raphé nuclei and microiontophoresis of 5-HT onto neurons of the suprachiasmatic nucleus produced similar effects on the firing rate of these hypothalamic cells, thus providing evidence that exogenously administered 5-HT could affect neurons in a manner similar to an endogenous synaptic transmitter.

In spite of this extensive neuropharmacological and biochemical research, the physiological roles of 5-HT in the brain have not been well elucidated, primarily because the techniques used have failed to provide sufficiently robust evidence of a causal relationship between the presence and/or release of 5-HT within the brain and physiological events. Nevertheless, there is an abundance of indirect evidence suggesting that 5-HT plays an important part in the normal function of a number of behavioral and homeostatic systems. Included among the latter is the system responsible for the regulation of body temperature.

A. Thermoregulatory effects of centrally administered 5-HT.

Brodie and Shore as early as 1957 theorized that, within the hypothalamus, 5-HT and NE acted in a mutually antagonistic manner to regulate various autonomic functions, including temperature regulation. This hypothesis was based on the known presence of 5-HT and norepinephrine (NE) in the hypothalamus (Amin et al., 1954; Vogt, 1954) and on the autonomic effects of certain drugs which, after systemic administration

either block receptors or alter the rate of release of monoamines within the CNS (Dasgupta and Werner, 1954; Shore et al., 1957). However, the first experimental evidence that 5-HT might be involved in thermoregulation was the discovery that a systemic injection of 5-hydroxytryptophan (5-HTP), the precursor of 5-HT, evoked a rise in body temperature in the rabbit (Horita and Gagerty, 1958). The demonstration in 1961 that either 5-HTP or 5-HT injected intracisternally in the rabbit produced a hyperthermia (Canal and Ornesi, 1961a) lends credence to the possibility that the hyperthermia produced by peripherally administered 5-HTP represented a central action of 5-HT. In the same year, Von Euler reviewed the available literature and discussed more extensively the possible roles of 5-HT and NE in thermoregulation. In 1963, Feldberg and Myers, on the basis of data obtained using the cat, adduced the formal hypothesis which has come to be described as the "monoamine theory of thermoregulation". These workers theorized that NE released within the hypothalamus would activate heat loss, whereas 5-HT released within this structure would stimulate heat production. Later, after observing the effects of 5-HT and NE injected directly into the cerebral ventricles and the anterior hypothalamus of the cat, Feldberg and Myers (1964, 1965) concluded that the anterior hypothalamus was the area where the balance in the release of these amines regulates the body temperature. Finally, in agreement with the findings in the cat, Myers and Yaksh (1969) reported that in the rhesus

monkey, an intracerebral (i.c.) injection of 5-HT caused a dose-dependent hyperthermia with the hyperthermic action of the amine being localized in the anterior hypothalamic/preoptic (AH/PO) area.

The thermoregulatory consequences of intracranial administration of monoamine oxidase inhibitors (MAOI) in the cat and dog support the postulated function of 5-HT in thermoregulation. Monoamine oxidase inhibitors increase the brain level of 5-HT in the cat and dog, whereas they produce no change in the brain level of NE (Vogt, 1959; Spector et al., 1960; Pscheidt et al., 1962; Spector, 1963). Since both the cat (vide supra) and the dog (Feldberg et al., 1967) experience a hyperthermia after an intraventricular injection of 5-HT, one would expect an intraventricular injection of MAOI to elicit an increase in body temperature. In the cat, each of four different MAOI injected into the lateral ventricles evoked a rise in body temperature (see Summers, 1973, for references). In the dog the MAOI, tranylcypromine, has been injected into the ventricles, and it produced a rise in colonic temperature (Feldberg et al., 1967). Moreover, when perfusing the third ventricle of the cat with the MAOI, tranylcypromine, the initial increase in 5-HT output from the third ventricle was associated with shivering and a rise in rectal temperature (El Hawary et al., 1967).

The evoked release of 5-HT from the anterior hypothalamus of the rhesus monkey by environmental cold stimulation constitutes a third major source of support for the idea

that 5-HT may be a neurotransmitter involved in heat production. Myers and Sharpe (1968) reported that environmental cooling could release a factor into the perfusate collected from the anterior hypothalamus of a donor monkey which could evoke a fever when infused into the anterior hypothalamus of a recipient monkey kept at room temperature. The recipient monkey did not develop a fever when the donor monkey was not subject to thermal stress. The perfusate from the anterior hypothalamus of the cooled monkey was reported to contain 4 to 24 times the amount of 5-HT found in the perfusate of a monkey not exposed to the cold (Myers et al., 1969), therefore, indicating that the indoleamine is released presynaptically within the anterior hypothalamus when heat production is required and that the amount of release is enough to initiate heat production.

In summary, there are three lines of evidence which indicate that in the anterior hypothalamus 5-HT may be a synaptic transmitter involved in initiating heat production: (1) the ability of an i.c. injection of 5-HT in the anterior hypothalamus to evoke a hyperthermia, (2) the ability of drugs that elevate levels of endogenous 5-HT in the brain to initiate a hyperthermia, and (3) the increased rate of release of 5-HT from the anterior hypothalamus in response to environmental cold stimulation.

However, the idea that an intracranial injection of 5-HT would elicit the same thermoregulatory effect in all species appears to be incorrect (Myers, 1970). Moreover,

the data regarding the thermoregulatory effects of centrally administered 5-HT within a given species is in some instances conflicting. For example, an intraventricular injection of 5-HT in the rat (Myers and Yaksh, 1968), mouse (Brittain and Handley, 1967), goat (Anderson et al., 1966), sheep (Bligh, 1966) and ox (Findlay and Robertshaw, 1967) produced a fall in body temperature. It has been generally assumed that these hypothermic responses were initiated by diffusion of 5-HT from the cerebroventricular fluid into the AH/PO region, because in the cat and monkey the hyperthermic action of 5-HT has been reported to be localized in the anterior hypothalamus and in the AH/PO area, respectively. However, 5-HT injected bilaterally into the AH/PO area of the rat produced only a dose-dependent rise in body temperature (Crawshaw, 1972). Therefore, 5-HT may be involved in both heat production and heat loss in the rat, with an area other than the AH/PO mediating the heat loss. In addition, since the AH/PO area of the mouse, goat, sheep and ox has never been examined for the ability to initiate a temperature change when injected with 5-HT, the possibility remains that in these species one area of the hypothalamus is evoking a hyperthermia whereas a different area within the CNS is eliciting the heat loss.

In the rabbit, the type of temperature response elicited by an intraventricular or an intracisternal injection of 5-HT was usually dose-dependent. High doses of 5-HT injected either intracisternally or intraventricularly usually

initiated a hyperthermia, whereas low doses of 5-HT usually evoked a hypothermia (Banerjee et al., 1970; Jacob and Girault, 1974). Cooper et al. (1965) reported that an injection of the indoleamine into the anterior hypothalamus of the rabbit would lower the body temperature if the animal was febrile; otherwise such injections were without effect. Based on these findings, Banerjee et al. (1970) theorized that 5-HT evoked a heat loss by acting on the anterior hypothalamus and that a different area within the central nervous system was responsible for the heat production elicited by a ventricular or a cisternal injection of 5-HT.

In the rhesus monkey, high doses of 5-HT injected into the AH/PO region have been reported to cause a fall in body temperature (Myers, 1968). However, because lower doses of 5-HT injected at the same site evoked only an increase in body temperature, Myers (1968) postulated that the hypothermia was evoked by the ". . . high concentration of this amine overloading the receptor sites in the anterior region and thus inhibiting the hyperthermic response." This idea is supported by the finding that low doses of 5-HT stimulate ganglionic cells in the inferior mesenteric ganglion of the cat, whereas higher doses inhibit the excitation of ganglion cells (Gyermek and Bindler, 1962).

Intraventricular injections of 5-HT have been reported to evoke a dose-dependent hypothermia in the cat (Kulkarni, 1967). However, Kulkarni (1967) dissolved 5-HT in distilled water, whereas Feldberg and Myers (1964), who reported that

intraventricular injections of 5-HT evoked a hyperthermia, dissolved 5-HT in 0.9% saline. The contradictory data led Banerjee et al. (1968) to repeat the intraventricular injections of 5-HT in the cat using distilled water and 0.9% saline as the solvents for 5-HT. They concluded that the difference in vehicles and dosage used by Feldberg and Myers (1964) and Kulkarni (1967) could explain the conflicting results, since the fall in body temperature was increased by dissolving 5-HT in water and/or by increasing the dosage of 5-HT. Furthermore, Banerjee et al. (1968) suggested that the hypothermic effect resulted from the "paralysis of the cells of the anterior hypothalamus" produced by the administration of "excessive" amounts of 5-HT or by dissolving a normal dose of 5-HT in distilled water, rather than normal saline. Distilled water was theorized to enhance the "paralysis of cells in the anterior hypothalamus" by releasing 5-HT. Thus, 5-HT dissolved in water could in theory elicit a hyperthermia at lower doses than when dissolved in 0.9% saline. This line of reasoning may explain why high doses of 5-HT injected into the medial preoptic area of the cat produced a hypothermia of more than 2^0 C (Jacobson, 1967). However, it does not explain why Kulkarni (1967) reported that 5-HT evoked a fall in body temperature with doses smaller than those shown by Feldberg and Myers (1964) and Banerjee et al. (1968) to produce a temperature increase.

As described previously, central injections of 5-HT have been shown to elicit both a hypothermia and a

hyperthermia in several species. Possibly, within these species, different mechanisms may be causing the hypothermia and the hyperthermia. In the rat and rabbit it seems that the hypothermia evoked by 5-HT is mediated by one region within the CNS and that the hyperthermia produced by this monoamine is mediated by a different region (vide supra, pp. 6 and 7). Although, in the monkey, a hypothermia can be initiated by an injection of 5-HT in the AH/PO, it has been suggested that the heat loss is due to high concentrations of 5-HT blocking the receptors involved in initiating the hyperthermia (Myers, 1968). Similarly, the hypothermia elicited by an intraventricular injection of 5-HT in the cat was theorized to be caused by high doses of 5-HT and/or the use of distilled water as the vehicle for 5-HT (Banerjee et al., 1968). However, the evidence in the cat does not totally support the conclusions of Banerjee et al. (1968). The evidence does suggest the possibility that one region within the CNS is responsible for the 5-HT induced hyperthermia and that a different region may be mediating the hypothermia elicited by a central injection of 5-HT. Furthermore, in the cat, only a few microinjection sites outside of the anterior hypothalamus have ever been tested for the ability to initiate a body temperature response to 5-HT (Feldberg and Myers, 1965). Therefore, because other areas of the cat's diencephalon have not been examined for sensitivity to 5-HT and because intraventricular injections of 5-HT will elicit a hypothermia at doses lower than those

used to evoke a hyperthermia, the possibility remains that one region of the cat brain may initiate only a fall in body temperature when injected with 5-HT.

B. Mediators of the hyperthermia evoked by central injections of 5-HT.

In spite of the evidence suggesting that 5-HT in the anterior hypothalamus may be a neurotransmitter involved in initiating heat production, no information as to the effectiveness of a specific 5-HT antagonist in preventing or reducing the hyperthermia initiated by 5-HT has appeared in the literature. A peripheral injection of cyproheptadine has been reported to antagonize the hyperthermia induced by either an intracisternal (Canal and Ornesi, 1961b) or an intraventricular injection of 5-HT (Jacob and Peindaries, 1973; Jacob and Girault, 1974) in the rabbit. However, cyproheptadine, in addition to being a 5-HT receptor antagonist, is known to have strong antihistaminic and anticholinergic activity (Stone et al., 1961; Page, 1968; Reizen, 1972). Consequently, part or all of the observed antagonism may not be due to the ability of cyproheptadine to block 5-HT receptors.

Feldberg and Myers (1963) reported that the hyperthermia elicited by 5-HT in the cat usually occurred ". . . in two peaks. There is first a transient, relatively quick rise, which is followed within one hour after the temperature has returned to normal by a second more gradual but prolonged

elevation of up to 2^0 C. It is sometimes more than 20 hours before the rectal temperature has returned to normal." An amine which is rapidly cleared from the brain (Tozer et al., 1966; Bulat and Supek, 1967; Myers et al., 1971; Hashi et al., 1972) would not be expected to mediate by way of a direct postsynaptic action a fever which is biphasic and of long duration. There is thus a need to consider the possibility that the hyperthermia evoked by centrally administered 5-HT is mediated by some mechanism other than an action of the amine at its post-synaptic receptor.

Trauma to the tissue surrounding the injection site could elicit the hyperthermia seen after an injection of 5-HT. Radiofrequency, thermal lesions in the anterior hypothalamus of the goat (Andersson et al., 1965) and electrolytic lesions in the anterior hypothalamus of the cat (Teague and Ranson, 1936) and rhesus monkey (Ranson et al., 1937) have been reported to cause a hyperthermia. In both types of lesions, the hyperpyrexia was believed to be due to trauma-induced stimulation of the tissue around the lesion because the hyperthermia decreased in several days and because increasing the size of the lesion induced a hypothermia (Ranson and Magoun, 1939; Keller, 1960). Additional support for the idea that the hyperthermia seen after an injection of 5-HT is evoked by trauma to the adjacent tissue is the finding that an i.c. injection of saline or artificial cerebrospinal fluid (ACSF) into the anterior hypothalamus of the cat can produce a fever (Jackson, 1967; Rudy and

Komiskey, unpublished observations).

Prostaglandins could be mediating the increase in body temperature seen after an i.c. injection of 5-HT in the cat. On a weight basis, PGE_1 is about a 1000 times more potent than the monoamines in eliciting a hyperthermic response when injected into the ventricles or the anterior hypothalamus of the cat (Feldberg and Saxena, 1971a; Milton and Wendlandt, 1971a; Feldberg and Milton, 1973). Piper and Vane (1971) stated that the "mammalian cell seems to disgorge prostaglandins at the slightest provocation". Even though prostaglandins do not appear to be stored within the body, they are nevertheless rapidly synthesized on demand (Vane, 1972). Although, it is not known whether prostaglandins can be released from brain as easily as they are from peripheral tissues, there is no evidence to the contrary. In any case, due to the properties of the prostaglandins just discussed, it has been suggested that prostaglandins may be mediating the hyperthermia which often develops after an injection of 0.9% saline or ACSF into the ventricles of an experimental animal (Feldberg and Saxena, 1971b; Milton and Wendlandt, 1971a). In support of this suggestion is the finding that an intraperitoneal injection of indomethacin, an inhibitor of prostaglandin synthesis (Vane, 1972), prevented the febrile response evoked by an i.c. injection of ACSF in the AH/PO of the cat (Rudy and Komiskey, unpublished observations).

Besides non-specific trauma of the injection evoking

prostaglandin release, it is possible that the 5-HT induced fever is due to a specific release of prostaglandins by 5-HT. Holmes (1970) reported that 5-HT caused a four-fold increase in the rate of release of prostaglandins into the perfused ventricular system of the dog. On the other hand, intraventricular infusions of epinephrine and norepinephrine had no effect on the rate of prostaglandin release into the cerebrospinal fluid. Whatever the mechanism(s) may be by which 5-HT causes the release of prostaglandins in the CNS, the involvement of prostaglandins in the hyperthermia evoked by an i.c. injection of 5-HT is indicated by the finding that an intraperitoneal injection of acetaminophen, a neuro-specific prostaglandin synthesis inhibitor, prevented or abolished the hyperthermic response produced by intraventricular injections of 5-HT in the cat (Milton and Wendlandt, 1971b).

C. <u>A putative cholinergic pathway mediating the hyperthermia induced by 5-HT</u>.

Based on the similarities in the responses produced by peripherally administered serotonergic and cholinomimetic drugs which pass through the blood brain barrier and the modification of these effects with cholinergic antagonists which also pass through the blood brain barrier, Brodie and Shore (1957) suggested that a cholinergic pathway mediated all the autonomic functions, including temperature changes initiated by endogenous 5-HT in the hypothalamus. Although

the only information which has appeared in the literature with regard to the possibility that a cholinergic pathway may be mediating the temperature changes evoked by 5-HT was obtained in the monkey, the evidence available strongly supports this concept. Myers and Yaksh (1969) proposed that a cholinergic pathway, originating in the hypothalamus, was mediating the heat production initiated by an i.c. injection of 5-HT in the monkey. This concept was based on the finding that 5-HT exerted a hyperthermic action only when injected into the AH/PO area of the rhesus monkey, whereas cholinomimetic substances evoked a sharp rise in body temperature at sites within and caudal to the AH/PO region. Additional experimental evidence for the concept was provided by the finding that peripheral cooling of the unanesthetized rhesus monkey increased the rate of 5-HT release in only the AH/PO area (Myers and Beleslin, 1971), whereas this cooling increased the rate of acetylcholine (ACh) release in and caudal to the AH/PO area (Myers and Waller, 1973). Finally, the idea that a cholinergic pathway may mediate the rise in body temperature initiated by an i.c. injection of 5-HT is further supported by a report that i.c. injections of 5-HT induced a release of ACh within the hypothalamus and thalamus during the 5-HT evoked hyperthermia in the pig-tailed monkey (Myers and Waller, 1975).

D. Statement of the problem.

As stated in the introduction, in the anterior

hypothalamus of several species, 5-HT may be a neurotransmitter involved in initiating heat production. However, in spite of all the evidence supporting this concept, no information has appeared in the literature indicating the effectiveness of a specific 5-HT receptor antagonist in preventing or reducing the hyperthermia evoked by a central injection of 5-HT. On the other hand, there is evidence suggesting that prostaglandins may be causing the hyperthermia observed after a central injection of 5-HT.

The hypothermia induced by an intraventricular injection of 5-HT in the cat has been suggested to be caused by high doses of the indoleamine (Banerjee et al., 1968). However the data do not support the above suggestion, since the lowest doses injected intraventricularly in the cat evoked a hypothermia (vide supra). Although the anterior hypothalamus has been examined for sensitivity to 5-HT, other areas in and outside the hypothalamus have not been examined for the ability to initiate a temperature response to a microinjection of 5-HT. Sympathomimetic amines and carbamylcholine have been reported to evoke a temperature response at microinjection sites outside of the hypothalamus in the cat (Rudy and Wolf, 1971, 1972). Therefore it is possible that one region within the diencephalon of the cat may initiate only a fall in body temperature when injected with 5-HT, whereas another region may produce only a rise when pharmacologically activated by the indoleamine.

The evidence obtained by Myers and coworkers indicates

that a cholinergic pathway is mediating the hyperthermia initiated by an i.c. injection of 5-HT in the AH/PO area of the monkey. It is possible that a cholinergic pathway is mediating the temperature changes evoked by i.c. injections of 5-HT in the cat. This idea is supported by the following findings: (1) in the cat, the hyperthermic action of 5-HT has been localized in the anterior hypothalamus (vide supra) and (2) cholinomimetic substances evoke an increase in body temperature at microinjection loci throughout the hypothalamus in the cat (Rudy and Wolf, 1972) similar to the monkey (Myers and Yaksh, 1969). On the other hand a cholinergic pathway may not be mediating the temperature changes evoked by 5-HT in the cat, since the hypothermia initiated in the cat and monkey by an i.c. injection of a cholinomimetic substance appears to be mediated by different areas of the hypothalamus. In the cat, only at loci within the anterior hypothalamus is a hypothermia the predominate temperature response elicited by cholinergic stimulation (Rudy and Wolf, 1972), whereas a central injection of a cholinomimetic substance evokes a hypothermia in the monkey only when injected into the junction of the posterior hypothalamus and the mesencephalon (Myers and Yaksh, 1969).

In view of the considerations discussed above, the objectives of the study were to determine:

(1) the involvement of 5-HT receptors and prostaglandins in the biphasic hyperthermia evoked by an i.c. injection of 5-HT

(2) the possibility that a specific diencephalic region(s) may be mediating the hypothermia observed after certain i.c. injections of 5-HT

(3) the nature of the involvement of synaptic 5-HT receptors in the hypothermia produced by an i.c. injection of 5-HT

(4) the possibility that an efferent cholinergic pathway may be mediating the body temperature changes evoked by an i.c. injection of 5-HT.

II. METHODS

A. General Methods

1. Experimental subjects

Fifteen mongrel cats of either sex obtained from the University of Wisconsin Animal Care Facility and weighing 2.5-4.5 kg served as experimental subjects. The cats were housed in individual cages at an ambient temperature of 22 ± 2.0^{0} C when not used in experiments. They were fed canned commercial cat food (Puss'n Boots) once daily, and water was available *ad lib*. except during experimental sessions. Lighting was controlled automatically on a 12-hour-on 12-hour-off schedule. Veterinary care was provided by the University of Wisconsin Medical School Animal Care Facility.

2. Surgical procedure

Cats were implanted with an array of 2 to 10 micro-injection guides distributed with an equal number on both sides of midline. The coordinates of the intended implantation sites were determined from the stereotaxic atlas of Snider and Niemer (1961). All surgery was performed under aseptic conditions.

Each cat was deprived of food and water 24 hours prior to surgery. The animal was anesthetized with pentobarbital sodium, 35 mg/kg, administered intravenously, and its head was placed in a Kopf stereotaxic instrument. The subject

was draped, the cranium exposed by a dorsal midline incision, and the field cleared of muscle and fascia. The craniotomy was made large enough to accomodate the entire array of microinjection guides. Stainless-steel machine screws were inserted into holes taped in the bone adjacent to the craniotomy. The dura was incised at the projected point of entrance of each guide and the entire array, held as a unit in a fixed configuration by a modified stereotaxic electrode carrier, was lowered into the brain so the tip of each guide was above the diencephalon or 1.5 mm above the intended sites of injection. Exposed dura was covered with Gelfoam® and the array was then fixed securely to the skull by flowing cranioplastic cement (L. D. Caulk Co., Milford, Delaware) around the screws and guides.

To protect the array from mechanical disturbance and to aid in maintaining a sterile preparation, a hollow polyethylene pedistal was placed around the array and partially filled with cranioplastic cement.

3. Experimental session protocol

The cats were allowed two weeks to recover from surgery before any experimentation began. Since cats were more calm after eating, they were fed immediately prior to the experimental session. In every subject, experiments at each microinjection site (except vehicle injections) were separated by an interval of at least 5 days in order to reduce any possibility of carry-over effects or the development of

tolerance. However, if an i.c. injection of 5-HT failed to elicit a temperature response within one hour of the injection, another site below a different guide cannula was sometimes screened for sensitivity to 5-HT, either on the same day or on the following day.

All experiments were performed with the cat maintained in a closed environmental chamber (2' x 3' x 1') which was, in turn, located within an isolation room kept at 20.0 $\pm$ 0.5^{0} C. A small fan (100 cfm) in the right corner of the chamber was used to circulate room air through the chamber. The subjects were restrained by a stock-like device and a thoracic harness which prevented them from displacing the thermistors and the microinjection tubing, but permitted the animal to assume a range of resting postures (Rudy, 1974). The cats were placed in the restraining device for 5 to 10 hours in most experiments. In each experiment, baseline data were obtained for at least one hour prior to any injection. Furthermore, an injection was not made until the colonic temperature varied less than 0.1^{0} C within 30 minutes.

4. Microinjection technique

Although microiontophoresis is the best available method for evaluating the qualitative effects of neurotransmitters on single neurons, the microinjection technique was used in this study to characterize central receptors. In the microiontophoretic technique, a small amount of ionized

substance is expelled into the immediate spatial environment of a neuron by passing an electrical current of the same charge through a drug-containing micropipette. However, microiontophoretic stimulation of a single neuron is usually incapable of initiating a physiological response. Therefore, one cannot determine which of the neurons examined in a neuronal region are involved in a particular physiological response using microiontophoresis.

A physiological response can readily be obtained using the microinjection technique. The microinjection technique consists of infusing a small volume (1.0 µl) of drug solution directly into brain tissue, thus affecting a group of neurons rather than a single neuron. Rudy and Wolf (1971, 1972) have shown that a dose-dependent temperature response can be obtained by microinjecting sympathomimetic amines and cholinergic agonists into the hypothalamus. One can then characterize the central receptor at which a suspected neurotransmitter acts by applying specific pharmacological agonists and antagonists in an attempt to mimic or diminish, respectively, the temperature response.

Drug solutions were injected through 28 gauge stainless-steel cannulae inserted through permanently implanted 22 gauge stainless-steel guides. When the guides were not being used for an i.c. injection, they were occluded with a solid stainless-steel stylet. In a typical microinjection, the injection cannula, connected to a short length of PE-10 polyethylene (PE) tubing, was filled with a drug solution.

A small air bubble (approximately 1 mm in length) was introduced into the PE tubing at the end opposite the injection cannula. A 10 µl Hamilton microliter syringe filled with 70% alcohol was connected to the tubing behind the bubble. The movement of the small air bubble permitted one to visualize the flow of the solution once the motorized microinjection pump was activated. The external surface of the guide and surrounding pedistal was cleaned with acetone. The occluding stylet was withdrawn from the guide and the injection cannula was then lowered with sterile forceps. Penetration beyond the desired depth was prevented by a collar soldered to the shank of the injection cannula. Once all parameters had again restabilized (usually less than 5 min.) the motorized microinjection pump, which lay outside the environmental chamber, was activated. Thus, the cat was neither handled at the time of an i.c. injection nor provided with any auditory or visual cues as to when the microinjection was performed.

In each experiment, an i.c. injection consisted of 1 µl of fluid delivered at a constant rate over a period of 2 minutes. The injection cannula was not removed until the end of the experimental session. Injections were first made 1.5 mm below the guide tip. Once all experiments at this depth had been completed, other depths were examined at 1 mm intervals until the base of the brain was reached. The microinjection cannula, the polyethylene microinjection tubing and microliter syringe were stored in 70% alcohol

when not in use.

Solutions for an i.c. injection were made by dissolving the drug in sterile artificial cerebrospinal fluid (ACSF) (Myers, 1971). The ACSF used had an ionic composition, osmolality, and pH similar to cerebrospinal fluid (see Appendix 1 for composition of ACSF). This was necessary in order to avoid nonspecific physiological effects and neuronal damage (Rech and Domino, 1959; Myers and Yaksh, 1971; Myers and Veale, 1971; Seoane and Baile, 1973). Sodium chloride was used to adjust the osmolality of the ACSF. Osmolality and pH determinations were made for each concentration of every agent used.

It was essential that the injected solution be both sterile and pyrogen-free because the agents were in many instances administered into pyrogen-sensitive regions. To minimize the chance of pyrogen contamination, all utensils used in preparing ACSF and all nonheat-labile constituents of the ACSF were dry heated at 190° C for 2 hours prior to use. The constituents of the ACSF were dissolved in sterile, pyrogen-free distilled water (for irrigation, U.S.P.). The resulting solution was forced through a Millipore filter (which had been autoclaved at 125° C and 20 lbs. pressure for 30 min.) of 0.22 μ average pore diameter into sterile, depyrogenated serum vials. The serum vials were sealed with sterile rubber stoppers, stored at 2° C and used within three weeks. The rubber stoppers were previously boiled for half an hour to remove surface wax and autoclaved at 125° C and

20 lbs. pressure for 30 minutes. When needed, the solution within a vial was brought to pH 7.3 by bubbling with 95% O_2-5% CO_2 for 10 minutes. The ACSF was then withdrawn from the vial and combined with the drug in a sterile volumetric flask. The resulting drug solution was then passed through a Millipore filter, received in a sterile, pyrogen-free, disposable syringe and used within two hours.

B. Recording and quantification of physiological responses.

1. Colonic temperature

The colonic temperature (T_c, in ^{0}C) was detected by a lubricated thermistor probe (#401, Yellow Springs Instrument Co. (YSI), Yellow Springs, Ohio) inserted 10 cm into the colon and taped to the base of the tail. The thermistor was connected to a YSI model 43TA telethermometer whose output was recorded by a dual-channel, continuous writeout potentiometric recorder or a scanning, multi-channel potentiometric recorder. The systems had a precision of $\pm 0.05^0$ C.

The maximum (T_c max., in ^{0}C), minimum (T_c min., in ^{0}C), maximum change (ΔT_c, in ^{0}C) and the initial rate of change ($i\dot{T}_c$, in ^{0}C/hour) in colonic temperature can be employed as measure of drug effectiveness. However, because the baseline rectal temperature varies between subjects, T_c max. and T_c min. observed after a drug treatment are poor quantifiers of drug effectiveness and were therefore not employed in this study.

The maximum change in colonic temperature ΔT_c, was one

measure used to quantify the thermoregulatory effects of the drug treatments used in this investigation. However, both the pharmacological potency and the rate of removal of an agent from an i.c. injection site will affect ΔT_c. Hence, because increasing the dosage would probably prolong the sojourn of the drug at an active site after an i.c. injection, one might expect ΔT_c to be dose-related. Since the duration of action of a drug at an injection site affects ΔT_c, it was not the only measure used to quantify the dose response data obtained with i.c. injections of 5-HT.

The initial rate of change in colonic temperature, $i\dot{T}_c$, is a better measure of a drug's potency, since it is relatively uninfluenced by the duration of action of a drug at an injection site. However, $i\dot{T}_c$, cannot always be measured accurately due to occasional short-term variations caused by movement of the thermistor probe within the colon or the passage of fecal material in the colon. Therefore, the rate of colonic temperature change, averaged over the first 50 percent of ΔT_c (50% $\Delta T_c/\Delta T$, in ^{o}C/hour), was also used to quantify the dose response data obtained with i.c. injections of 5-HT.

2. Ear skin temperature

The temperature of the dorsal surface of the ear (T_e) was taken as an index of cutaneous vasomotor tone. A small piece of transparent tape (Scotch brand magic tape No. 810) was placed on the shaved area of the ear prior to the

beginning of each experimental session. Then, a small disc-shaped thermistor probe (YSI, type 427) was affixed acutely to the center of the dorsal surface of the ear with Marlin Ileo Cement. The transparent tape prevented the Marlin Ileo Cement from irritating the ear. Ear skin temperature was recorded in the same manner as T_c (vide supra, p. 19).

3. Visually observed thermoregulatory and nonthermoregulatory parameters.

In each experimental session, the animal was observed for the presence of huddling, piloerection, or shivering. Although the primary concern was the participation of serotonin in thermoregulation, serotonin has been implicated to be involved in sleep (Jouvet, 1968; Koella et al., 1968), emotions (Stevens et al., 1967; Wise et al., 1970) and in hyperreactivity (Conner et al., 1970). Therefore, the behavior of each cat was followed closely and recorded during and between experimental sessions. The thermoregulatory parameters and behavior of the animals during the experimental sessions was observed through the clear plexiglass top of the environmental chamber by way of a one-way mirror located in a wall of the isolation room.

C. Experimental Design

1. Dose-response relationships

Different doses of 5-HT were injected into i.c. injection sites in which the indoleamine evoked either a

biphasic hyperthermia or a hypothermia followed by a rise in body temperature in order to determine if the evoked temperature response was dose dependent. At i.c. injection sites where 5-HT initially elicited a hypothermia a "standard" concentration of 5-HT was administered in alternation with the other doses tested in order to determine if the sensitivity of the injection site remained constant. At i.c. injection sites where 5-HT evoked a biphasic rise in temperature, the effects of only two doses were evaluated. The lower concentration of the indoleamine was injected first, followed by the higher concentration in the next experimental session.

2. Antagonist studies

Antagonists were injected either intraperitoneally or into i.c. injection sites prior to an i.c. injection of 5-HT or an appropriate vehicle control solution. A control injection of 5-HT was performed at each i.c. injection site 6 days before and 6 days after an experiment in which the effect of an antagonist on a 5-HT response was examined. To control for any vehicle effects or effects of an intraperitoneal injection, during the subsequent experimental session the vehicle for each antagonist was always administered prior to the control 5-HT i.c. injection at the same pretreatment time that the particular antagonist was given. An antagonist was considered effective only if, when compared to the immediately preceding as well as the subsequent control

injection of 5-HT, it significantly altered the temperature response to 5-HT.

3. Data analysis

The mean and standard error of the mean (S.E.M.) of the temperature response produced by control injections of 5-HT and by the indoleamine after pretreatment with an antagonist were computed. The effects of the antagonists were analyzed by a one-way analysis of variance with repeated measures (Winer, 1962). The significance of difference between individual means were determined using Duncan's new multiple range test (Steel and Torrie, 1969). A P value of <0.05 was taken as evidence of a significant alteration of the effect of 5-HT by an antagonist.

D. Histology

When the experiments in a cat were completed, 1 µl of India ink was injected into the deepest brain site which had received an i.c. injection, and the animal was sacrificed with pentobarbital sodium. The thorax was then opened and the descending aorta and heart clamped. The jugular veins were sectioned and the brain perfused through the ascending aorta with 0.9% saline followed by buffered 10% formalin. The head was placed in the stereotaxic headholder and, using stereotaxically guided knife cuts, a block of tissue containing the region of interest was removed. The block of tissue was first placed in a formalin-sucrose solution

(10% formalin containing sucrose 30 gm/100 ml) for at least 48 hours. The block was then placed successively in 5% and 10% gelatin at 40° C (24 hours in each concentration). Finally, the block was placed in the formalin-sucrose solution again until it sank to the bottom of the solution (about 48 hours).

Frozen sections were then cut at 40 micra and stained by a modification of the method of Klüver and Barrera (1953). The sites of injection were determined by microscopic examination and represented on coronal and sagittal projections of the cat brain.

E. Drugs Used

Atropine sulfate and atropine methyl nitrate (Sigma Chemical Co., St. Louis, Mo.) were dissolved in sterile, 0.9% saline (Travenol Laboratories, Inc., Morton Grove, Ill.). Creatinine sulfate (CS), 5-hydroxytryptamine creatinine sulfate (5-HT) and l-hyoscyamine hydrochloride (Sigma Chemical Co.) were dissolved in sterile ACSF. Cyproheptadine, indomethacin (Merck Sharp and Dohme, Rahway, N.J.), acetaminophen (Sigma Chemical Co.) and metergoline (A. H. Robins, Richmond, Va.) were suspended in 1% carboxymethycellulose (CMC) (Merck and Co. Inc., Rahway, N.J.). Methysergide hydrogen maleate (Sandoz Pharmaceuticals, Hanover, N.J.) was dissolved in either 5% polyethylene glycol 400 (PEG 400) (Baker Chemical Co., Phillipsburg, N.J.) or warm, sterile 0.9% saline (Travenol Laboratories,

Inc.) when administered peripherally to cats. When injected directly into the brain, methysergide was dissolved in sterile ACSF at the molar concentrations (M) stated in the text. Finally, cinanserin and SQ10,631 (E. R. Squibb and Sons, Inc., Princeton, N.J.) were dissolved in 0.9% saline.

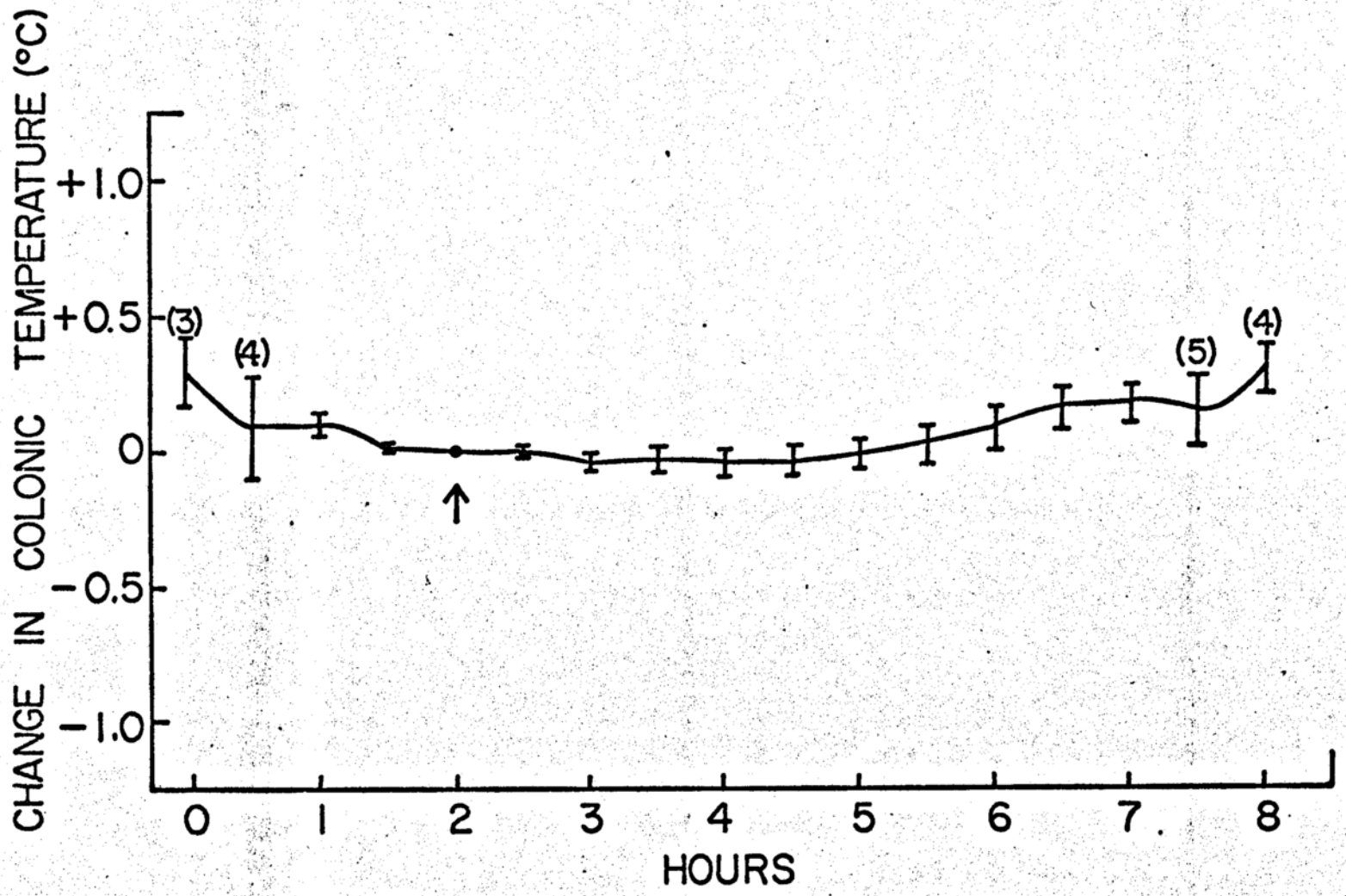
All the drugs injected intraperitoneally, except indomethacin, were administered in volume of 0.2 ml/kg. Indomethacin was injected intraperitoneally in a volume of 0.25 ml/kg.

III. RESULTS

A. Characteristics of the thermoregulatory changes evoked by intracerebral injections of 5-HT.

Many of the experiments to be described required that a cat be restrained at an ambient temperature of 20° C for up to 10 hours. To ascertain the effect on body temperature of this procedure, seven of the 15 cats used in the study were restrained for 7-8 hours at 20° C using the method previously described (p. 20). The chamber door was opened briefly at time 2 hours to simulate the opening of the chamber carried out when a drug treatment was administered. The results are shown in Figure 1. It can be seen that briefly opening the chamber door did not cause a change in mean core temperature and, with the exception of the first and last 30 minutes, that core temperature did not vary appreciably throughout the session. Within the first 30 minutes after opening and closing the door of the environmental chamber, the mean maximal change in colonic temperature was 0.02° C (S.D. = 0.05° C). If spontaneous fluctuations in core temperature under the conditions described are normally distributed, less than 1 percent of such fluctuations can be expected to fall outside the limits of three standard deviations of the mean (±0.15° C), and less than 0.1 percent will exceed the limits of ±5 S.D. (±0.25° C) (Steel and Torrie, 1960). It was therefore assumed in the experiments to be described that a change in colonic temperature of 0.25° C or more

Figure 1. Mean change in colonic temperature from baseline (point at the arrow) of cats restrained within a closed environmental chamber maintained at 20.0 $\pm$ 0.5^{0} C. To control for any thermoregulatory effects produced by opening the top cover of the environmental chamber during the experimental sessions when the injection cannula was normally inserted, the top cover of the environmental chamber was opened for 2 minutes at the arrow. Vertical bracketed lines represent the standard errors of the means. Except where indicated (numbers in parentheses) means are based on data from 7 cats.


CHANGE IN COLONIC TEMPERATURE (°C)
+1.0
+0.5
0
−0.5
−1.0
(3)
(4)
(5)
(4)
0
1
2
3
4
5
6
7
8
HOURS

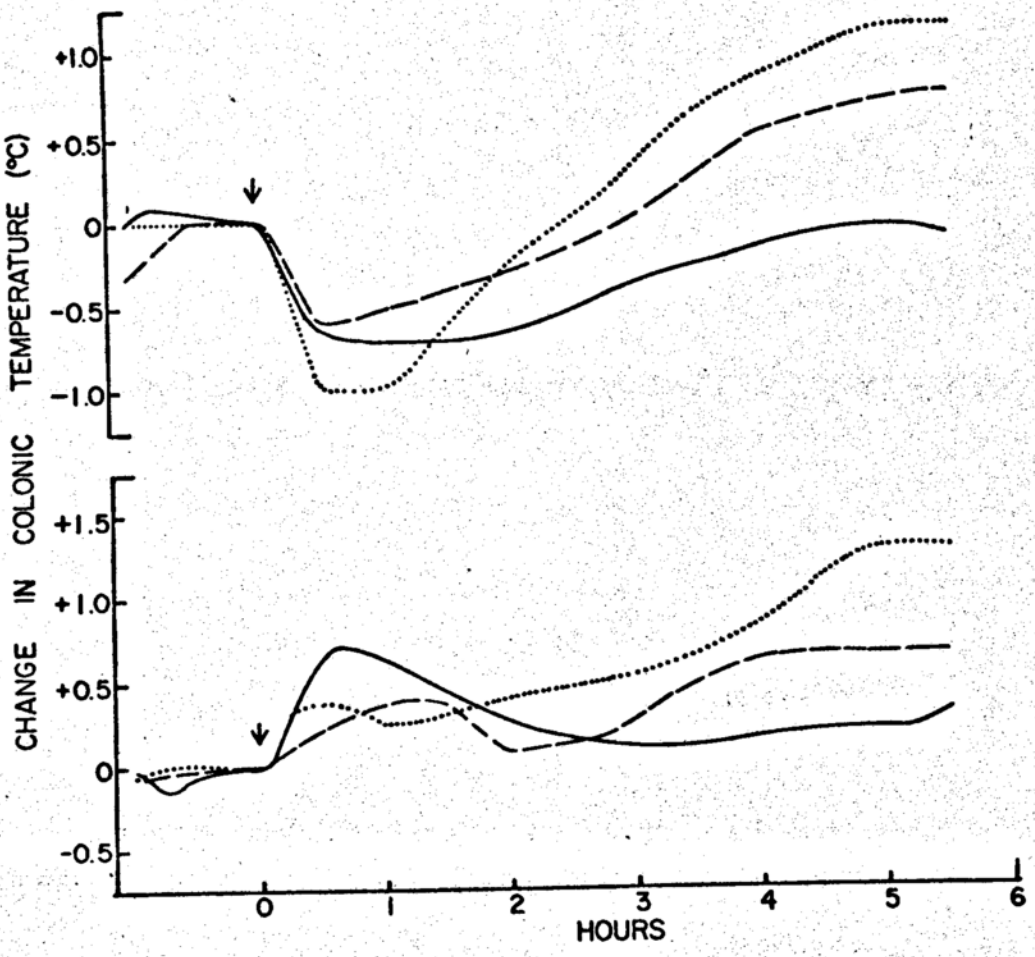
occurring within 30 minutes of an i.c. injection or other experimental treatment represented a significant thermoregulatory response to the treatment.

5-Hydroxytryptamine, 0.01 or 0.03 M, was injected into a total of 160 i.c. loci in a total of 15 cats. Within the first 30 minutes after injection of 5-HT into 115 of these sites, there was no appreciable change in body temperature. When injected into the remaining 45 loci, 5-HT caused either an initial rise (19 sites) or fall (26 sites) in body temperature. Both types of initial effect were frequently followed by a secondary temperature increase. In many instances, injections which produced no initial change in body temperature were also followed by a delayed hyperthermia. Several examples of the two basic types of colonic temperature change observed after i.c. injections of 5-HT are presented in Figure 2.

Prior to an i.c. injection, the skin of the ear pinna was always vasoconstricted, ear temperature (T_e) being at most 2^0 C above ambient. No decrease in T_e was observed during the initial hyperthermia produced by a 5-HT injection. However, during a 5-HT-induced fall in body temperature, vasodilation lasting up to two hours frequently occurred. The response consisted of a large T_e increase of 7-12^0 C. Intermediate levels of vasodilation were not observed. Once the vasodilation had occurred, it proceeded rapidly to the maximum change, as did the subsequent vasoconstriction.

An intense tremor always accompanied the initial rise

Figure 2. Representative examples, obtained from 6 cats, of the change in body temperature produced by i.c. injections of 0.03 M 5-HT. Each curve represents the response of a single cat to 5-HT injected at the arrow. Responses were characterized by either an initial decrease (TOP) or increase (BOTTOM) in colonic temperature.


CHANGE IN COLONIC TEMPERATURE (°C)
+1.0
+0.5
0
-0.5
-1.0
+1.5
+1.0
+0.5
0
-0.5
0
1
2
3
4
5
6
HOURS

in body temperature evoked by an i.c. injection of 5-HT. Although only an analysis of the frequency characteristics of physiological shivering and the 5-HT induced tremors can determine unequivically whether the tremor seen in response to an i.c. injection of 5-HT is truly shivering, the tremor had the visual appearance of an intense cold-induced shivering. The tremor began over the hind legs during the last 30 seconds of the injection or immediately after the injection and sometimes spread to the whole body musculature. It decreased in intensity as colonic temperature approached its maximum level and was no longer visible after the peak of the hyperthermia had been achieved. The tremor was never observed during the hypothermia induced by 5-HT.

Panting was never observed. Piloerection could not be quantified reliably by visual inspection.

B. Nonthermoregulatory effects elicited by intracerebral injections of 5-HT, and by peripheral and intracerebral injections of various other agents.

1. Intracerebral injections of 5-HT

The cats were usually quiet and calm after i.c. injections of 5-HT. However, restlessness, vocalization or sedation were occasionally observed. These behaviors have been reported to occur after intraventricular injections of 5-HT (Feldberg and Myers, 1964; Kulkarni, 1967; Banerjee et al., 1968).

2. Intraperitoneal injections of various agents

Injections of cinanserin (Rubin et al., 1964; Rosen and Cohen, 1973), SQ10,631 (E. R. Squibb and Sons, Inc., personal communication, 1974) and metergoline (Beretta et al., 1965; Haigler and Aghajanian, 1974), produced no noticeable behavioral or autonomic effects. Injections of the 5-HT antagonist, methysergide (Fanchamps et al., 1960; Gyermek, 1961; Cottrell, 1970) usually produced restlessness. In addition the drug always caused mydriasis and often produced vomiting within 5-10 minutes of the injection. The cats appeared even more restless after injections of cyproheptadine (Stone et al., 1961; Page, 1968; Reizen, 1972). In contrast, the cats became very calm and quiet after treatment with either indomethacin or the neuro-specific prostaglandin inhibitor, acetaminophen (Vane, 1972). Injections of atropine methyl nitrate, a quaternary muscarinic cholinergic antagonist (Innes and Nickerson, 1970), produced mydriasis but no other observable effect. The tertiary muscarinic cholinergic antagonist, atropine sulfate (Innes and Nickerson, 1970) invariably produced episodes of excitement associated with visual scanning of the environment and struggling.

3. Intracerebral injections of antagonists

Injections of methysergide or the muscarinic cholinergic antagonist, l-hyoscyamine (Domino and Hudson, 1959; Innes and Nickerson, 1970) usually provoked no noticeable

behavioral effect. However, on one occasion, l-hyoscyamine elicited a coordinated rage response which included hissing, biting, yawling and struggling.

C. Dose-response relationships for the initial and delayed rises in colonic temperature produced by intracerebral injections of 5-HT.

The initial, short-lasting colonic temperature increase elicited by i.c. injections of 5-HT was found to be dose-dependent. As illustrated in Figure 3, both the maximum colonic temperature increase and the 50% $\Delta T_c/\Delta T$ of the initial rise evoked by 5-HT increased in a dose-dependent manner at the four sites tested. In addition, the sensitivity to 5-HT as shown in Figure 3 varied with the site of injection.

The maximum increase in body temperature during the second phase, defined as the rise in temperature above baseline which occurred after the first peak but within four and one-half hours of an i.c. injection of 5-HT, did not show any dose-response relationship at the doses tested (Figure 4). Feldberg and Myers (1965) indicated that the magnitude of the secondary hyperthermia evoked by 5-HT was greater than the initial increase in body temperature. However, in this study, at the 15 sites where an injection of 0.01 or 0.03 M 5-HT elicited an initial and secondary hyperthermia, the mean temperature increase of the first phase did not differ significantly ($P > 0.1$) from the mean rise in

Figure 3. Log dose-response curves describing the maximum increase in body temperature and the maximum rate of increase in body temperature (50% $\Delta T_c/\Delta T$) during the first phase of the hyperthermia produced by i.c. injections of 5-HT. Each solid circle represents the response to a single injection of 5-HT. Each open circle represents the mean response elicited during the same time period by an injection of 0.015 molar creatinine sulfate (CS) at one of the sites and an injection of 0.046 molar CS at three of the sites. Vertical bracketed lines represent standard errors of the means.

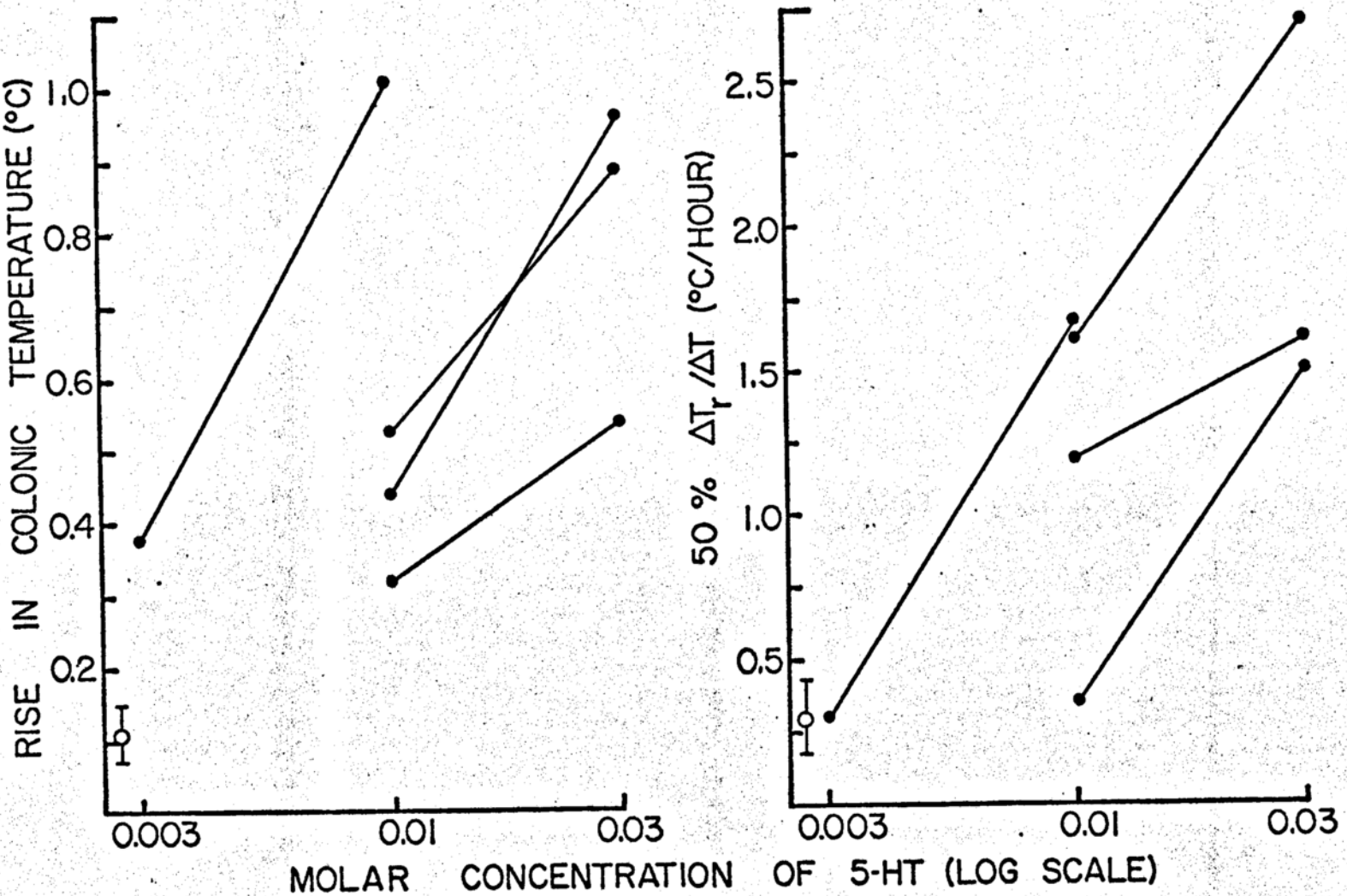
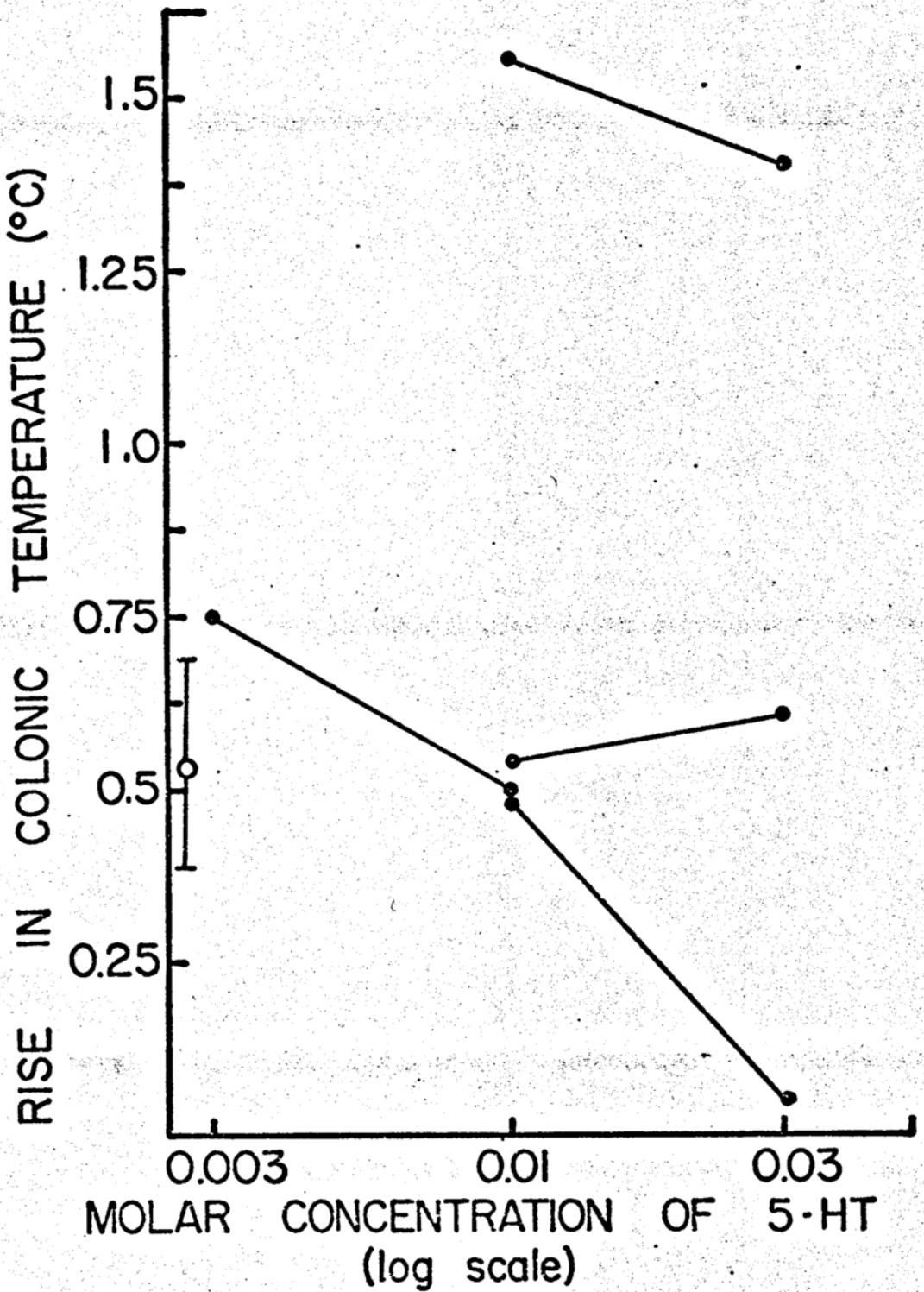

RISE IN COLONIC TEMPERATURE (°C)
1.0
0.8
0.6
0.4
0.2
50 % $\Delta T_r / \Delta T$ (°C/HOUR)
2.5
2.0
1.5
1.0
0.5
0.003
0.01
0.03
MOLAR CONCENTRATION OF 5-HT (LOG SCALE)

Figure 4. Log dose-response curves showing the maximum increase in body temperature during the second phase of the hyperthermia produced by i.c. injections of 5-HT. Each solid circle represents the response to a single injection of 5-HT. Each open circle represents the mean response elicited during the same time period by an injection of 0.015 molar CS at one of the sites and an injection of 0.046 molar CS at three of the sites. The vertical bracketed line represents the standard error of the mean.


RISE IN COLONIC TEMPERATURE (°C)
1.5
1.25
1.0
0.75
0.5
0.25
0.003
0.01
0.03
MOLAR CONCENTRATION OF 5-HT
(log scale)

temperature during the second phase (Table 1). In addition, the maximum rise in colonic temperature observed during the second phase evoked by a microinjection of 5-HT at 15 sites did not differ significantly ($P > 0.1$) from the rise in colonic temperature elicited during the same time period by a vehicle injection at the sites (Table 1). In contrast, the maximum rise in colonic temperature during the first phase of the hyperthermia evoked by 5-HT at the 15 active sites did vary significantly ($P < 0.02$) from the rise in colonic temperature produced during the same time period by an injection of the vehicle at the sites (Table 1).

D. Antagonism of the biphasic hyperthermia induced by 5-HT.

The possible involvement of endogenous prostaglandins and of 5-HT receptors in eliciting the biphasic hyperthermia elicited by 5-HT was examined using prostaglandin synthesis inhibitors and 5-HT receptor antagonists.

1. Prostaglandin synthesis inhibitors

Five cats, in which an i.c. injection of 0.03 M 5-HT evoked a biphasic hyperthermia, were pretreated with indomethacin, 10 mg/kg i.p., 3 hours prior to an i.c. injection of 5-HT. Indomethacin produced a hypothermia (about 0.5^{0} C) which had stabilized when 5-HT was injected. Figure 5 shows the effect of indomethacin on the 5-HT evoked biphasic rise in colonic temperature in one of the five animals. As shown in Table 2, indomethacin produced a significant reduction in

TABLE 1

The initial and the delayed hyperthermia produced by 5-HT or control solutions.

Treatment	n[c]	FIRST PHASE: Maximum ΔT_c (°C) Mean ± S.E.M.	FIRST PHASE: Comparison of means[b]	SECOND PHASE[a]: Maximum ΔT_c (°C) Mean ± S.E.M.	SECOND PHASE[a]: Comparison of means[b]
0.015 M CS[d]	5	0.12 ± 0.03	P < 0.02	0.45 ± 0.15	P > 0.1
0.01 M 5-HT		0.53 ± 0.12	P > 0.1	0.36 ± 0.07	
0.046 M CS or ACSF[e]	10	0.07 ± 0.02	P < 0.01	0.56 ± 0.15	P > 0.1
0.03 M 5-HT		0.56 ± 0.08	P > 0.1	0.80 ± 0.17	

[a] Mean maximum increase in colonic temperature after the first phase and within 4.5 hours after an injection of 5-HT.

[b] A paired-sample Student's t-test was used to determine the significance of difference between means connected by solid lines.

[c] Number of different microinjection sites tested with each set of treatments.

[d] Creatinine sulfate.

[e] ACSF was injected at five of the sites and 0.046 M SC at the other five sites.

Figure 5. The effect of pretreatment with indomethacin on the two phases of the rise in colonic temperature elicited by an i.c. injection of 5-HT. The treatments were administered in the order in which they appear in the figure legend. Agents were injected intraperitoneally (i.p.) or i.c. at the arrows. The vehicles for indomethacin and 5-HT were 1% CMC and ACSF, respectively.

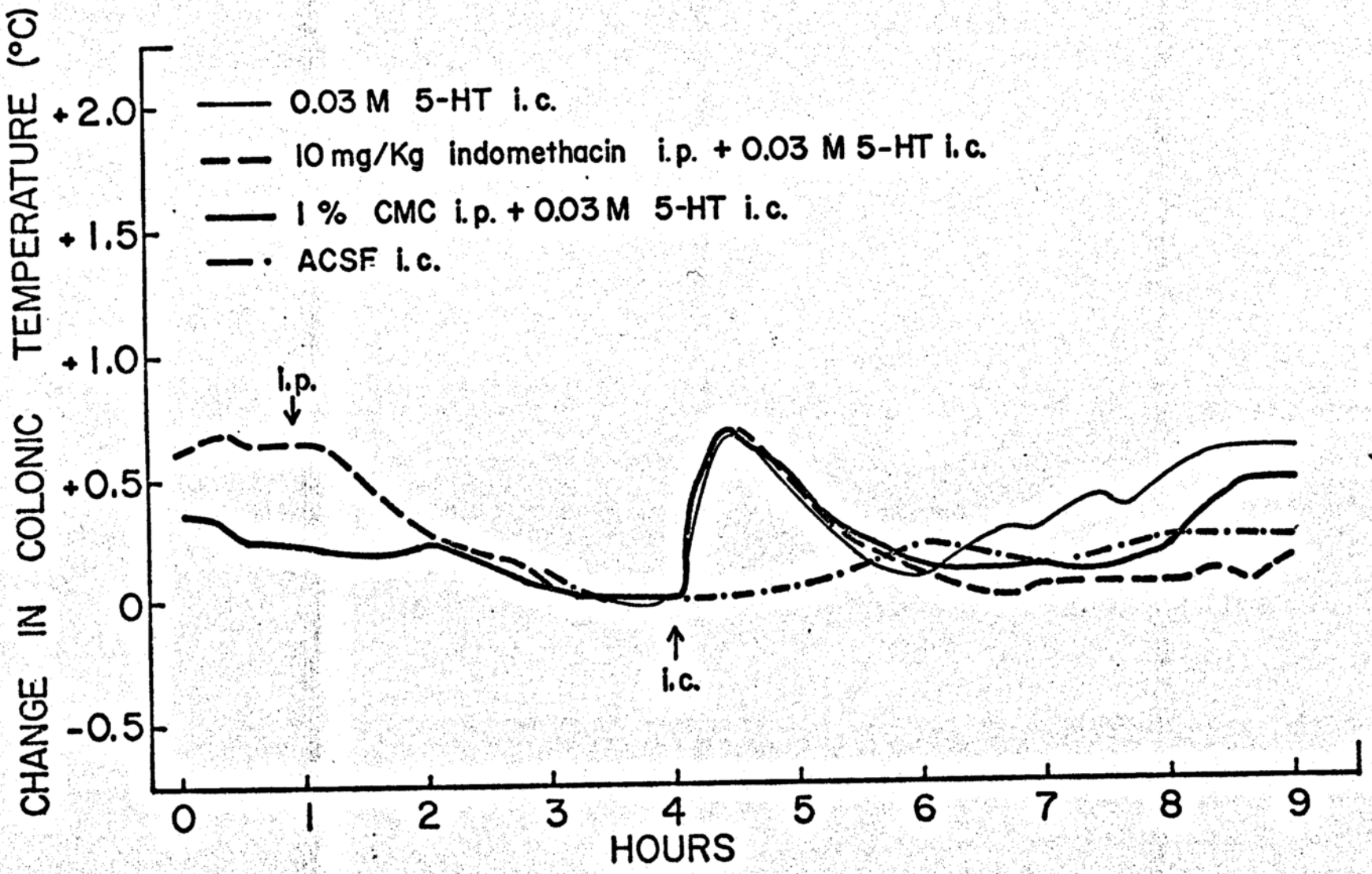

CHANGE IN COLONIC TEMPERATURE (°C)
+2.0
+1.5
+1.0
+0.5
0
-0.5
0.03 M 5-HT i.c.
10 mg/Kg indomethacin i.p. + 0.03 M 5-HT i.c.
1 % CMC i.p. + 0.03 M 5-HT i.c.
ACSF i.c.
i.p.
i.c.
0
1
2
3
4
5
6
7
8
9
HOURS

TABLE 2

Effect of an intraperitoneal injection of indomethacin or methysergide on the two phases of the rise in colonic temperature elicited by an intracerebral injection of 5-HT.[a]

FIRST PHASE[b]

ACSF or CS[c]	4	5	1	3	2
	methysergide+5-HT	5%PEG400+5-HT	5-HT	1%CMC+5-HT	indomethacin+5-HT
0.07±0.03	0.13±0.10	0.36±0.10	0.39±0.08	0.39±0.10	0.40±0.08

SECOND PHASE[d]

2	3	ACSF or CS	4	5	1
indomethacin+5-HT	1%CMC+5-HT		methysergide+5-HT	5%PEG400+5-HT	5-HT
0.10±0.04	0.37±0.07	0.54±0.14	0.66±0.19	0.67±0.09	0.74±0.15

The data presented represent the responses to an injection of 0.03 M 5-HT, 0.046 M CS, or ACSF in one locus in each of five cats. Indomethacin 10 mg/kg or its vehicle, 1%CMC, was injected intraperitoneally three hours prior to an i.c injection of 0.03 M 5-HT. Methysergide 2mg/kg or its vehicle, 5%PEG400, was injected intraperitoneally two hours prior to an i.c. injection of 0.03 M 5-HT.

[a]Each cat received the entire series of treatments. The numbers above the treatments represent the order in which the treatments were administered. The position of the ACSF and CS injections in the treatment sequence varied among animals and is therefore not assigned a treatment number. Any two means not underscored by the same line are significantly different ($p < 0.05$).

[b]Mean maximum increase in colonic temperature (°C)±S.E.M. occurring during the first rise in colonic temperature after an injection of 5-HT or during the same time period after an injection of either CS or ACSF.

TABLE 2 (Continued)

[c]Creatinine sulfate.

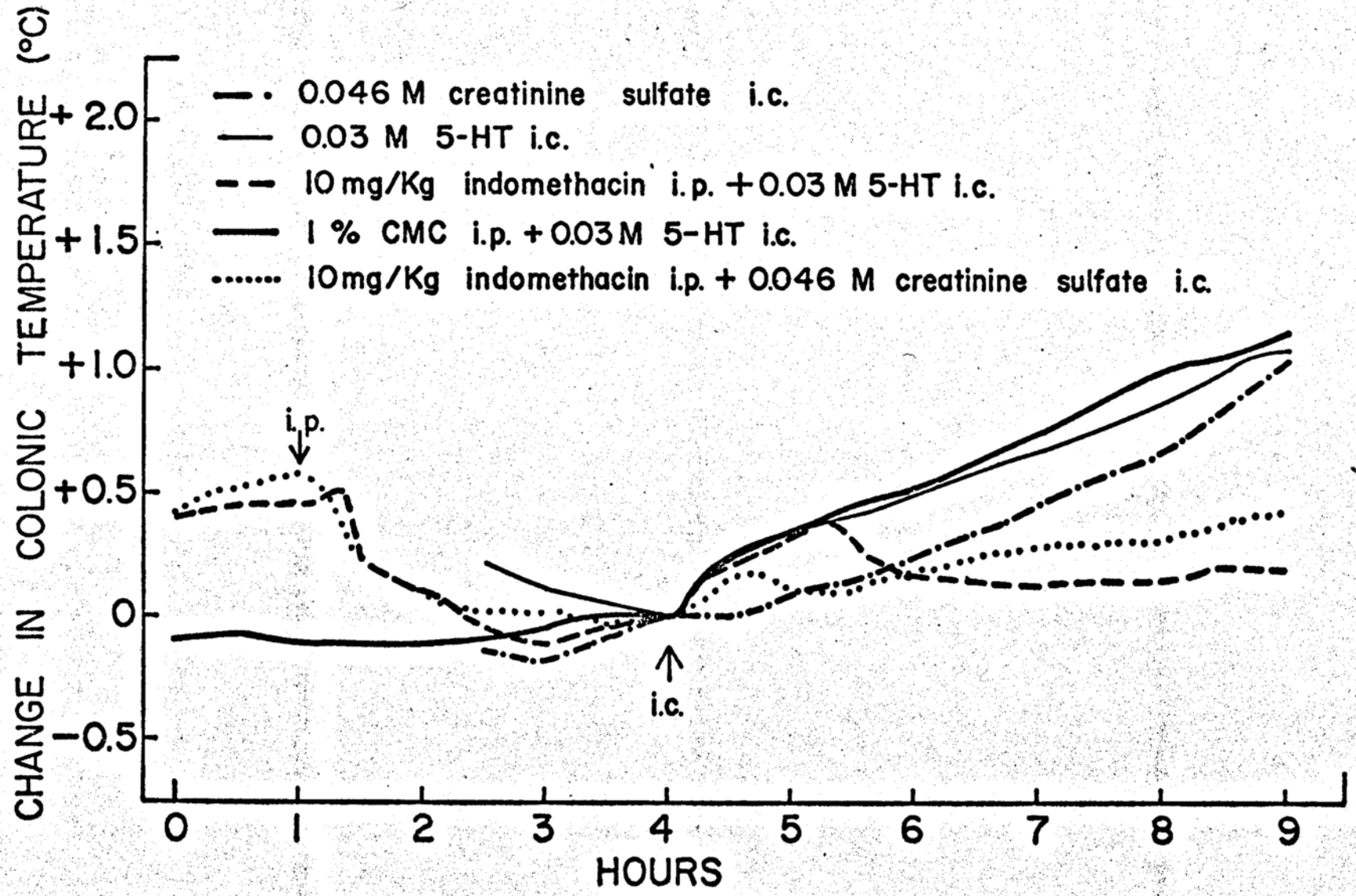
[d]Mean-maximum increase in colonic temperature (^{o}C)$\pm$S.E.M. occurring either after the first rise in colonic temperature and within 4.5 hours after an injection of 5-HT, or within 4.5 hours after an injection of CS or ACSF.

the rise in temperature during the second phase but it had no effect on the rise in temperature which occurred during the first phase. The apparent effectiveness of 1% CMC (treatment #3) in reducing the second phase may be a consequence of a prolonged action of the indomethacin administered 6 days earlier (treatment #2).

At two of the 19 i.c. injection sites where 5-HT originally elicited biphasic rises in colonic temperature, after 1-2 injections the hyperthermia changed to a monophasic rise in body temperature. Figure 6 illustrates the effect of indomethacin on a monophasic rise in body temperature elicited by 5-HT at one of the sites which originally initiated a biphasic rise in body temperature. Indomethacin did not prevent the initial rapid rise in body temperature but did prevent the gradual continuation of the rise. The latency of the second phase of the biphasic hyperthermia which was initially produced by 5-HT at the site had therefore decreased and as a result a monophasic hyperthermia was observed.

The time allowed the initial rapid rise to develop to its maximum after subsequent injections of 5-HT was the time required by the indoleamine to evoke its maximum rise in body temperature when injected 3 hours after indomethacin, 10 mg/kg i.p. In subsequent experiments at these two sites, the maximum rise in temperature which occurred within 4½ hours of the 5-HT microinjection was considered the maximum rise of the second phase.

Figure 6. The effect of pretreatment with indomethacin on a monophasic rise in colonic temperature produced by an i.c. injection of 5-HT. The treatments were administered in the order in which they appear in the figure legend. Agents were injected intraperitoneally (i.p.) or i.c. at the arrows. The vehicle for indomethacin was 1% CMC. The injection of 0.046 molar CS was used as a vehicle control for 5-HT.


0.046 M creatinine sulfate i.c.
0.03 M 5-HT i.c.
10 mg/Kg indomethacin i.p. + 0.03 M 5-HT i.c.
1 % CMC i.p. + 0.03 M 5-HT i.c.
10mg/Kg indomethacin i.p. + 0.046 M creatinine sulfate i.c.
CHANGE IN COLONIC TEMPERATURE (°C)
+2.0
+1.5
+1.0
+0.5
0
−0.5
i.p.
i.c.
0
1
2
3
4
5
6
7
8
9
HOURS

In a few experiments acetaminophen was injected intraperitoneally before a microinjection of 5-HT. Acetaminophen, 50 or 100 mg/kg produced a fall in body temperature which had stabilized at about 0.5° C below baseline within 3 hours of the injection. Acetaminophen (50 mg/kg or 100 mg/kg) was administered 3 hours before an i.c. injection of 5-HT. In a single experiment, the 50 mg/kg dose did not alter the first rise in body temperature initiated by 5-HT, whereas the second rise in body temperature was increased. In contrast, in two experiments acetaminophen (100 mg/kg) reduced by one-half the maximum rise in body temperature produced by 5-HT during the first phase , but it did not alter the second phase.

2. 5-Hydroxytryptamine antagonists

Several 5-HT receptor antagonists were available for use in this portion of the study, including metergoline, SQ10,631, cinanserin, cyproheptadine and methysergide. As the number of 5-HT-sensitive loci available was small and the number of injections into each site which could be performed without a decrement in site sensitivity was limited, it was decided to utilize extensively only one of these antagonists. In preliminary experiments, it was found that metergoline, SQ10,631 and cinanserin failed to affect the 5-HT-induced hyperthermia or affected it in an inconsistent manner. Both cyproheptadine and methysergide reduced preferentially the initial phase of the hyperthermia. As

indicated previously, cyproheptadine is not entirely selective in its blockade of 5-HT receptors. Therefore, methysergide was selected as the 5-HT receptor antagonist to be used in the majority of the remaining experiments.[1]

To the same five cats and using the same five injection sites as in the indomethacin experiments, methysergide, 2 mg/kg i.p., was administered 2 hours prior to an i.c. injection of 5-HT. Methysergide usually affected body temperature, causing either a small rise or fall which had stabilized at the new level when 5-HT was injected. Figure 7 illustrates the effect of methysergide on the 5-HT evoked hyperthermia in one of the 5 animals. Methysergide produced a significant reduction of the first phase but did not alter the second phase of the hyperthermia (Table 2). In addition a peripheral injection of 5% PEG 400, the vehicle used for methysergide in the above experiments, when administered 2 hours prior to an i.c. injection of 5-HT, did not affect either the first or the second phase of the hyperthermia.

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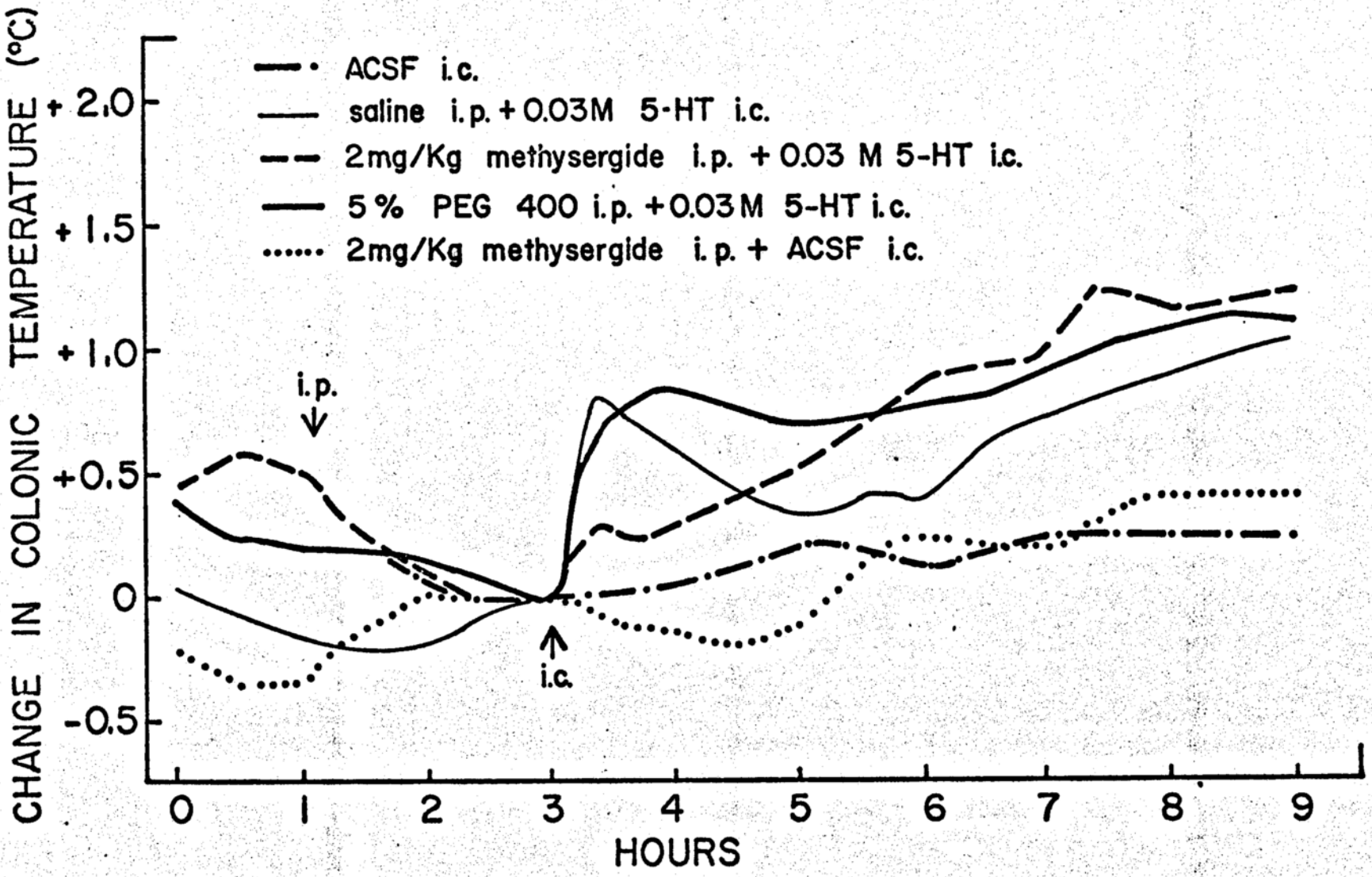
[1] In preliminary experiments, metergoline, SQ10,631, cinanserin, cyproheptadine, and methysergide were administered intraperitoneally to cats prior to an injection of 5-HT. All the 5-HT receptor antagonists produced a small fall in body temperature (0.25-0.50° C). Metergoline, 1 mg/kg, administered 1.5 hours or 2 hours prior to a microinjection of 5-HT either potentiated or antagonized the first phase of the hyperthermia without affecting the second phase. SQ10,631, 2 or 5 mg/kg, administered 60 minutes prior to an i.c. 5-HT, did not affect either phase of the hyperthermia. Cinanserin, 4.5 or 10 mg/kg, administered 60-90 min. prior to i.c. 5-HT also failed to affect either phase. Cyproheptadine, 2 mg/kg, given 90 min. before i.c. 5-HT, reduced the first phase by about 40% but did not alter the second phase. Methysergide, 2 mg/kg, 120 min. before 5-HT, produced an effect similar to that of cyproheptadine.

Figure 7. The effect of pretreatment with methysergide on the two phases of the rise in colonic temperature elicited by an i.c. injection of 5-HT. The treatments were administered in the order in which they appear in the figure legend. Agents were injected intraperitoneally (i.p.) or i.c. at the arrows. The vehicles for methysergide and 5-HT were 5% PEG 400 and ACSF, respectively.


ACSF i.c.
saline i.p. + 0.03M 5-HT i.c.
2mg/Kg methysergide i.p. + 0.03 M 5-HT i.c.
5% PEG 400 i.p. + 0.03 M 5-HT i.c.
2mg/Kg methysergide i.p. + ACSF i.c.
CHANGE IN COLONIC TEMPERATURE (°C)
+2.0
+1.5
+1.0
+0.5
0
-0.5
i.p.
i.c.
0
1
2
3
4
5
6
7
8
9
HOURS

It is possible that the antagonism of the first phase of the hyperthermia initiated by 5-HT with an intraperitoneal injection of methysergide may be due to an action of the antagonist, at sites other than those where 5-HT causes a hyperthermia. Therefore, methysergide was administered into the microinjection sites prior to a 5-HT injection in an attempt to antagonize the first phase of the hyperthermia. One microinjection site in each of three cats where 5-HT evoked a biphasic hyperthermia, was pretreated with an i.c. injection of 0.02 M methysergide 15 minutes prior to an injection of 0.01 or 0.03 M 5-HT. Figure 8 shows the effect of an injection of methysergide on the biphasic rise in colonic temperature evoked by 0.01 M 5-HT. The data from the three sites injected are summarized in Table 3. As can be seen, methysergide significantly reduced the magnitude of the first phase and potentiated the second phase of the hyperthermia.

Muscarinic stimulation of certain sites in the rostral hypothalamus of the cat can evoke a hyperthermia (Blanton et al., 1974). Intracerebral injections of 5-HT could possibly act by causing the release of endogenous ACh (Harry, 1963) or directly stimulating muscarinic receptors. To examine the possibility that methysergide blocks the initial phase of the hyperthermia evoked by 5-HT through an anticholinergic mechanism, 0.01 M l-hyoscyamine was injected into the same three sites mentioned directly above 20 minutes prior to 5-HT. This dose of l-hyoscyamine has been shown to reduce significantly the hyperthermia produced by the

Figure 8. The effect of pretreatment with an i.c. injection of methysergide on the two phases of the hyperthermia elicited by an i.c. injection of 5-HT. The injection of methysergide was administered at the first arrow 15 minutes before injecting 5-HT or CS into the site (second arrow). Creatinine sulfate and 5-HT were only injected i.c. at the second arrow. The treatments were administered in the order in which they appear in the figure legend. The injection of CS was used as a vehicle control for 5-HT.

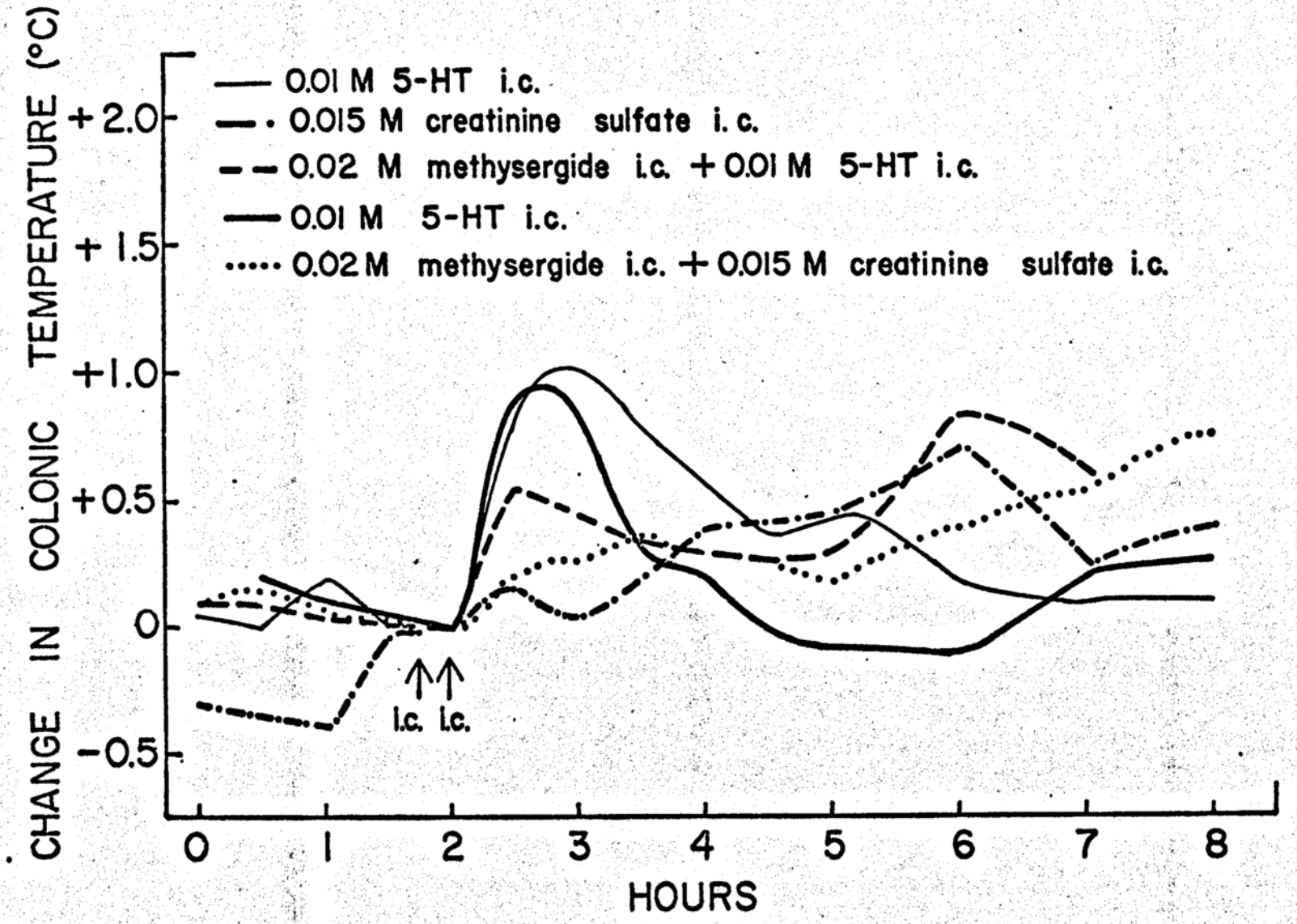

0.01 M 5-HT i.c.
0.015 M creatinine sulfate i. c.
0.02 M methysergide i.c. + 0.01 M 5-HT i.c.
0.01 M 5-HT i.c.
0.02 M methysergide i.c. + 0.015 M creatinine sulfate i.c.
CHANGE IN COLONIC TEMPERATURE (°C)
+2.0
+1.5
+1.0
+0.5
0
-0.5
i.c. i.c.
0
1
2
3
4
5
6
7
8
HOURS

TABLE 3

Effect of a preceding intracerebral injection of methysergide or hyoscyamine on the two phases of the rise in colonic temperature elicited by an intracerebral injection of 5-HT.[a]

FIRST PHASE[b]

7		2	6	4	5	3	8	1
hyoscyamine+CS[c]	CS[c]	methysergide +5-HT	methysergide +CS	hyoscyamine +5-HT	5-HT	5-HT	5-HT	5-HT
0.04±0.04	0.10±0.03	0.22±0.17	0.22±0.06	0.46±0.22	0.48±0.18	0.54±0.21	0.63±0.11	0.71±0.24

SECOND PHASE[d]

3	4	5	7	8		1	6	2
5-HT	hyoscyamine +5-HT	5-HT	hyoscyamine +CS	5-HT	CS	5-HT	methyser-gide+CS	methyser-gide+5-HT
0.40±0.21	0.45±0.26	0.52±0.29	0.56±0.22	0.62±0.27	0.66±0.16	0.72±0.36	0.75±0.29	0.98±0.21

The data represent the responses to an i.c. injection of 5-HT or CS obtained at one locus in each of three cats. An i.c. injection of 0.02 M methysergide or 0.02 M hyoscyamine was administered fifteen and twenty minutes, respectively, prior to an i.c. injection of either 0.01 M 5-HT or 0.015 M CS at one injection site. At the other two sites 0.02 M methysergide and 0.02 M hyoscyamine were administered fifteen and twenty minutes, respectively, prior to an i.c. injection of 0.03 M 5-HT or 0.046 M CS.

[a]Each cat received the entire series of treatments. The numbers above the treatments represent the order in which the treatments were administered. The position of the ACSF or CS injections in the treatment sequence varied among animals and is therefore not assigned a treatment number. Any two means not underscored by the same line are significantly different ($p < 0.05$).

[b]Mean maximum increase in colonic temperature (^{o}C)±S.E.M. occurring during the first rise in colonic temperature after an injection of 5-HT or during the same time period after an injection of CS.

[c]Creatinine sulfate.

[d]Mean maximum increase in colonic temperature (^{o}C)±S.E.M. occurring either after the first rise in colonic temperature and within 4.5 hours after an injection of 5-HT or within 4.5 hours after an injection of CS.

intracerebral injection of 0.03 M carbamylcholine in the cat (Rudy, Komiskey and Lau, unpublished observations). An example of the effect of 0.02 M l-hyoscyamine on a biphasic rise in body temperature evoked by 0.01 M 5-HT is presented in Figure 9. The hyoscyamine did not affect either phase of the hyperthermia elicited by 0.01 or 0.03 M 5-HT (Table 3).

E. Cholinergic pathway mediating biphasic hyperthermia.

Atropine sulfate and atropine methyl nitrate were used to investigate the possibility that a muscarinic cholinergic pathway could be mediating the biphasic rise in body temperature evoked by an i.c. injection of 5-HT in the cat. Cats in which 5-HT evoked a biphasic rise in body temperature were pretreated with atropine sulfate (2 mg/kg, i.p.) or with atropine methyl nitrate (2 mg/kg, i.p.) between 90 and 120 minutes prior to an i.c. injection of 5-HT. Figure 10 shows the effect of atropine sulfate on a biphasic rise in temperature evoked by 0.03 M 5-HT. The results are summarized in Table 4. Atropine sulfate significantly reduced the first phase, whereas atropine methyl nitrate produced a small but significant increase in the first phase of the hyperthermia initiated by 0.01 M 5-HT. Neither atropine sulfate nor atropine methyl nitrate significantly affected the second phase of the hyperthermia elicited by 0.01 M 5-HT.

As previously indicated (Table 3 and Figure 9) at the same microinjection sites in which an i.c. injection of 0.02 M methysergide significantly reduced the first rise in

Figure 9. The effect of pretreatment with an i.c. injection of l-hyoscyamine on the two phases of the hyperthermia elicited by an i.c. injection of 5-HT. The injection of l-hyoscyamine was administered at the first arrow 20 minutes before injecting 5-HT or CS into the site (second arrow). Creatinine sulfate and 5-HT were only injected i.c. at the second arrow. The treatments were administered in the order in which they appear in the figure legend. The injection of 0.015 molar CS was used as a vehicle control for 5-HT.

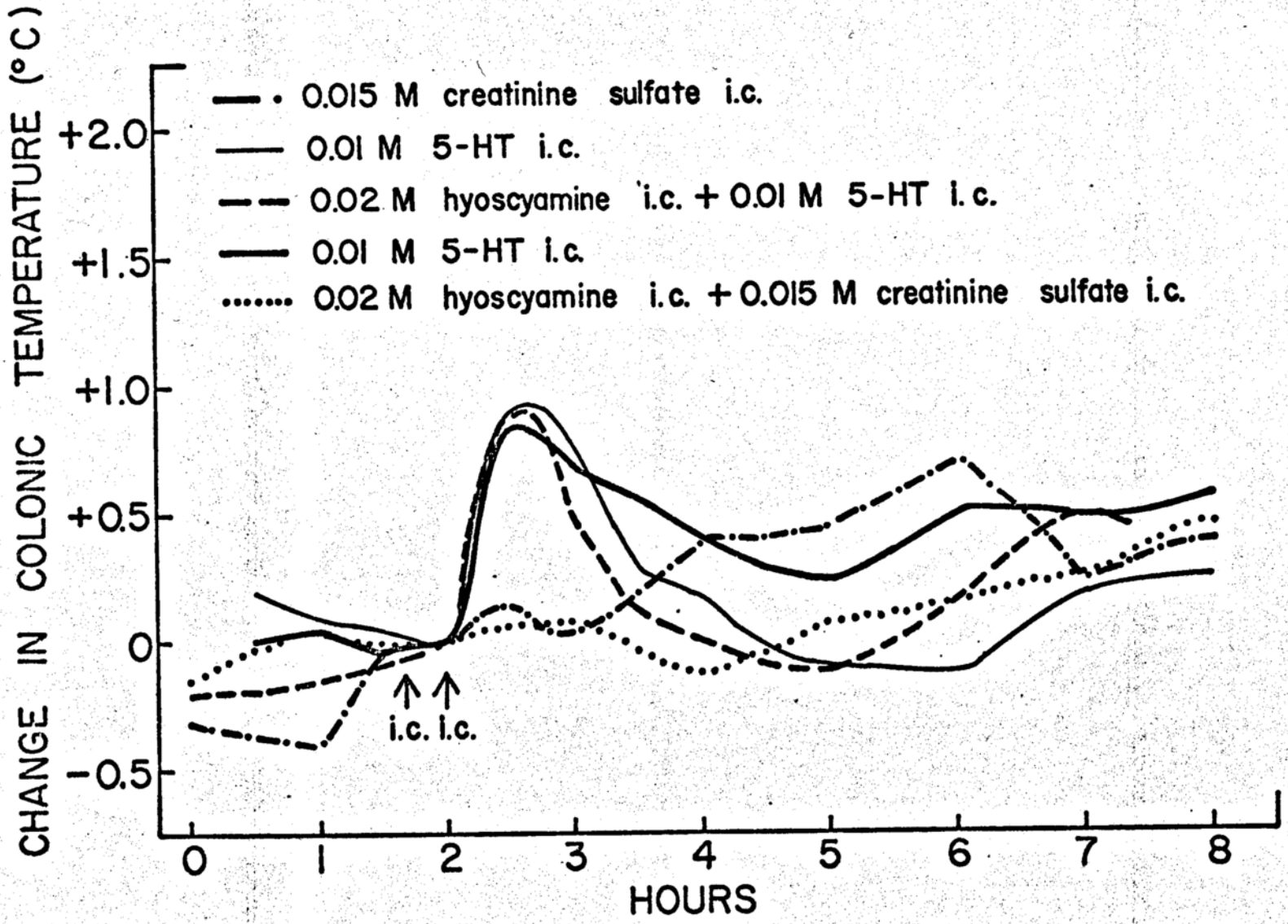

0.015 M creatinine sulfate i.c.
0.01 M 5-HT i.c.
0.02 M hyoscyamine i.c. + 0.01 M 5-HT i.c.
0.01 M 5-HT i.c.
0.02 M hyoscyamine i.c. + 0.015 M creatinine sulfate i.c.
CHANGE IN COLONIC TEMPERATURE (°C)
+2.0
+1.5
+1.0
+0.5
0
-0.5
i.c. i.c.
0
1
2
3
4
5
6
7
8
HOURS

Figure 10. The effect of pretreatment with an intraperitoneal injection of atropine sulfate on the two phases of the rise in colonic temperature evoked by an i.c. injection of 5-HT. The treatments were administered in the order in which they appear in the figure legend. Agents were injected intraperitoneally (i.p.) or i.c. at the arrows. The vehicles for atropine sulfate and 5-HT were 0.9% saline and ACSF, respectively.

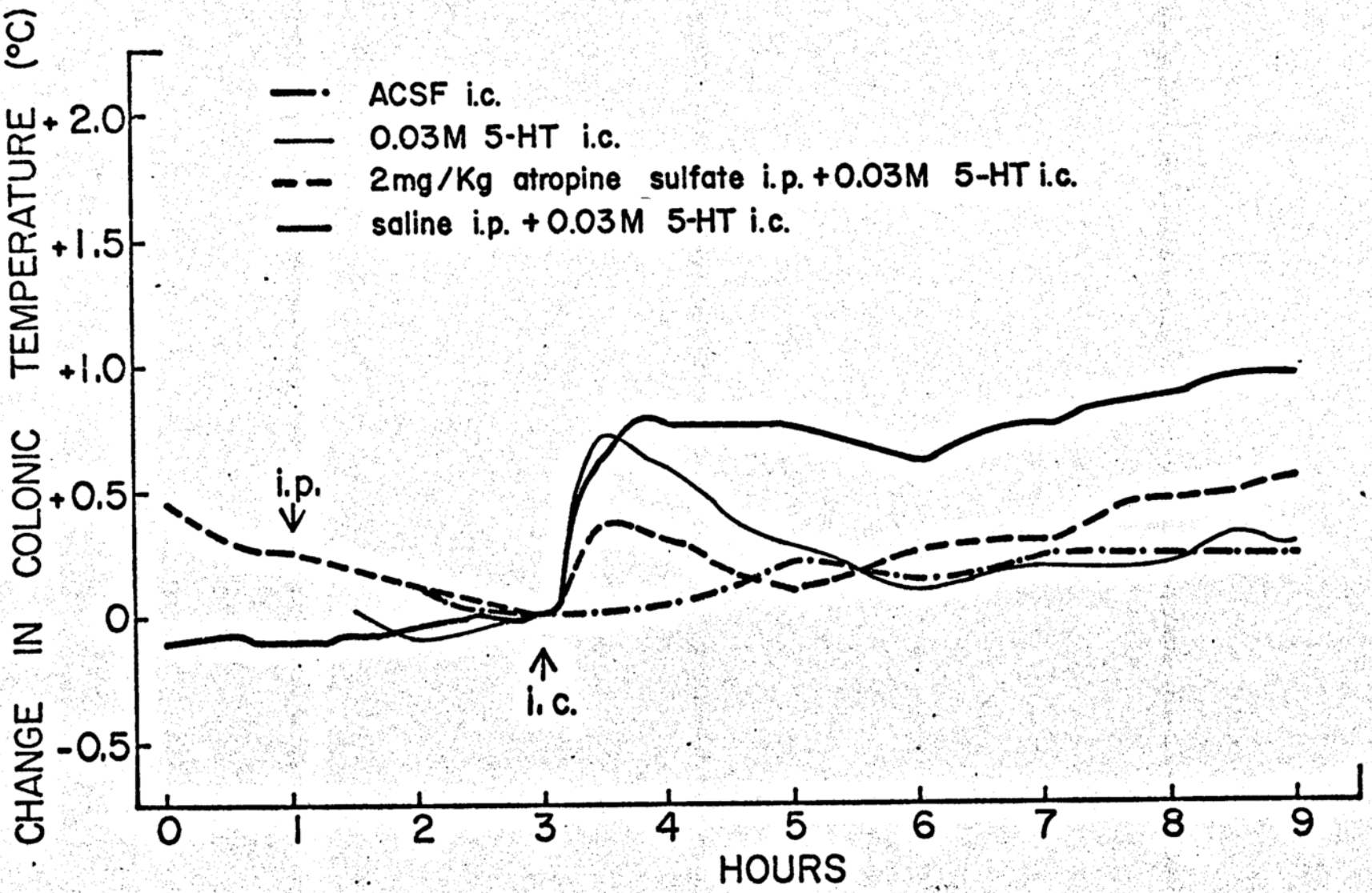

ACSF i.c.
0.03M 5-HT i.c.
2mg/Kg atropine sulfate i.p. + 0.03M 5-HT i.c.
saline i.p. + 0.03M 5-HT i.c.
CHANGE IN COLONIC TEMPERATURE (°C)
+2.0
+1.5
+1.0
+0.5
0
-0.5
i.p.
i.c.
0
1
2
3
4
5
6
7
8
9
HOURS

TABLE 4

Effect of an intraperitoneal injection of atropine sulfate or atropine methyl nitrate on the two phases of the hyperthermic response evoked by an intracerebral injection of 5-HT.[a]

FIRST PHASE[b]

CS[c]	2 atropine sulfate +5-HT	5 saline +5-HT	3 saline +5-HT	1 5-HT	4 atropine methyl nitrate +5-HT
0.05±0.03	0.18±0.02	0.34±0.10	0.35±0.09	0.41±0.03	0.44±0.20

SECOND PHASE[d]

2 atropine sulfate +5-HT	1 5-HT	CS	3 saline +5-HT	5 saline +5-HT	4 atropine methyl nitrate +5-HT
0.10±0.10	0.42±0.04	0.44±0.20	0.56±0.33	0.86±0.35	1.17±0.58

The data presented represent the responses to an i.c. injection of 0.01 M 5-HT or 0.015 M CS in one locus in each of three cats. Atropine sulfate, 2 mg/kg, atropine methyl nitrate, 2mg/kg, or an equivalent volume of normal saline were injected intraperitoneally between 1 1/2 and 2 hours prior to an i.c. injection of 0.01 M 5-HT.

a,b,c and d

As in table 3.

body temperature evoked by 0.01 or 0.03 M 5-HT, an i.c. injection of 0.02 M l-hyoscyamine did not significantly affect either phase of the hyperthermia initiated by 0.01 or 0.03 M 5-HT.

F. Dose response relationships for the fall and the delayed rise in colonic temperature evoked by 5-HT.

The initial fall in colonic temperature elicited by i.c. injections of 5-HT appears to be dose-dependent (Figure 11). However, the 50% $\Delta T_c/\Delta T$ of the initial fall sometimes decreased at the higher doses of 5-HT (Figure 11). In addition, the sensitivity of a microinjection site to 5-HT varied with the site of injection.

The magnitude of the hyperthermia which followed the initial fall in body temperature produced by an i.c. injection of 5-HT did not show any dose-response relationship at the doses tested (Figure 12). Moreover, the magnitude of the delayed rise did not differ significantly ($P > 0.05$) from the rise in colonic temperature elicited during the same time period by a vehicle injection at the same sites (Table 5). The maximum fall in colonic temperature evoked by 5-HT did, however, vary significantly ($P < 0.01$) from the fall in colonic temperature produced during the same time period by a vehicle injection (Table 5).

Figure 11. Log dose-response curves describing the maximum fall in body temperature and the maximum rate of fall in body temperature (50% $\Delta T_c/\Delta T$) produced by i.c. injections of 5-HT. Each solid circle represents the response to a single injection of 5-HT except at the highest concentration injected into each site. Each solid circle at the highest concentration of 5-HT injected into each site represents the mean response of 2 to 4 control injections of the indoleamine. Each open circle represents the mean response elicited during the same time period by an injection of 0.046 molar CS at two of the sites and an injection of ACSF at three of the sites. Vertical bracketed lines represent the standard error of the mean.

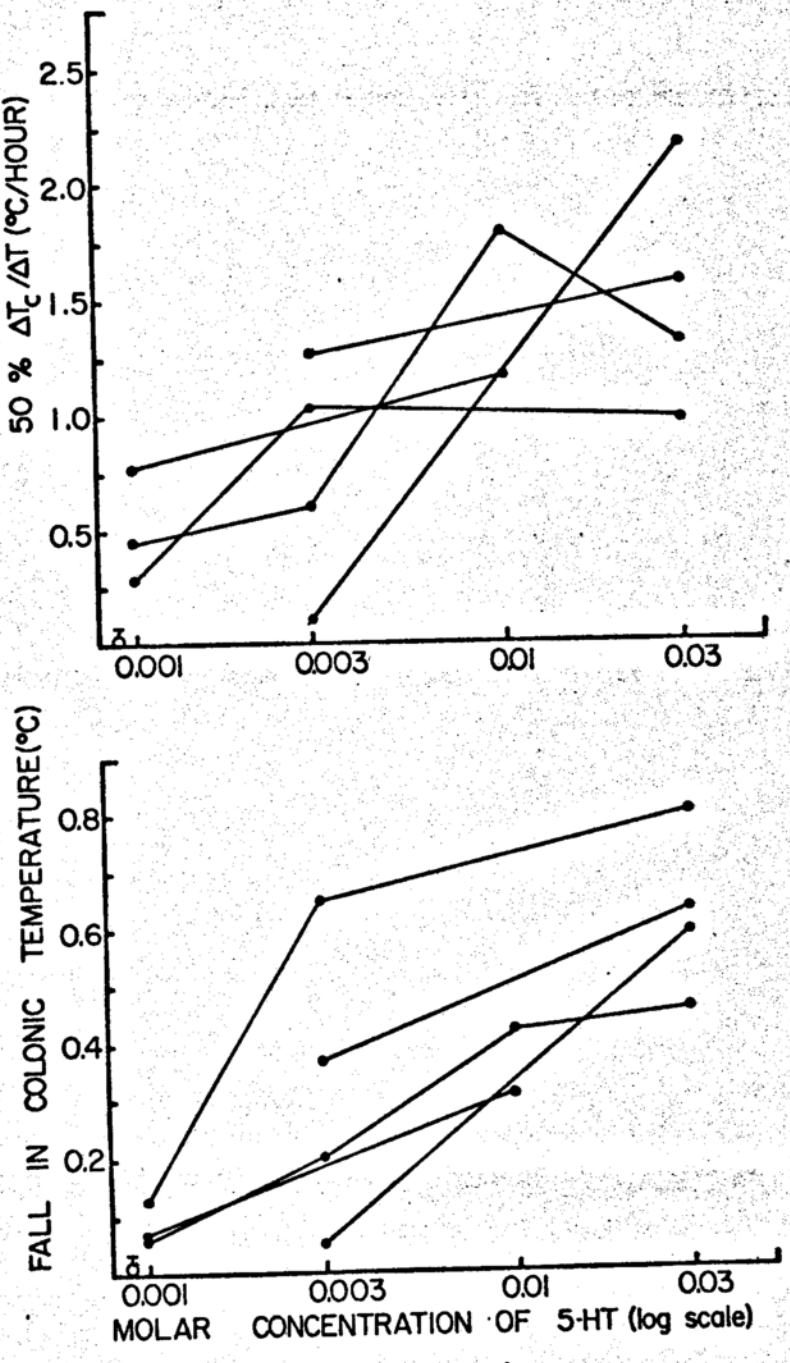

50 % ΔTc/ΔT (°C/HOUR)
2.5
2.0
1.5
1.0
0.5
0.001
0.003
0.01
0.03
FALL IN COLONIC TEMPERATURE(°C)
0.8
0.6
0.4
0.2
0.001
0.003
0.01
0.03
MOLAR CONCENTRATION OF 5-HT (log scale)

Figure 12. Log dose-response curves showing the maximum increase in body temperatures during the hyperthermia following the fall in temperature produced by i.c. injections of 5-HT. Each solid circle represents the response to a single injection of 5-HT except at the highest concentration injected into each site. Each solid circle at the highest concentration of 5-HT injected into each site represents the mean response of 2 to 4 control injections of the indoleamine. Each open circle represents the mean response elicited during the same time period by an injection of 0.046 molar CS at two of the sites and an injection of ACSF at three of the sites. The vertical bracketed line represents the standard error of the mean.

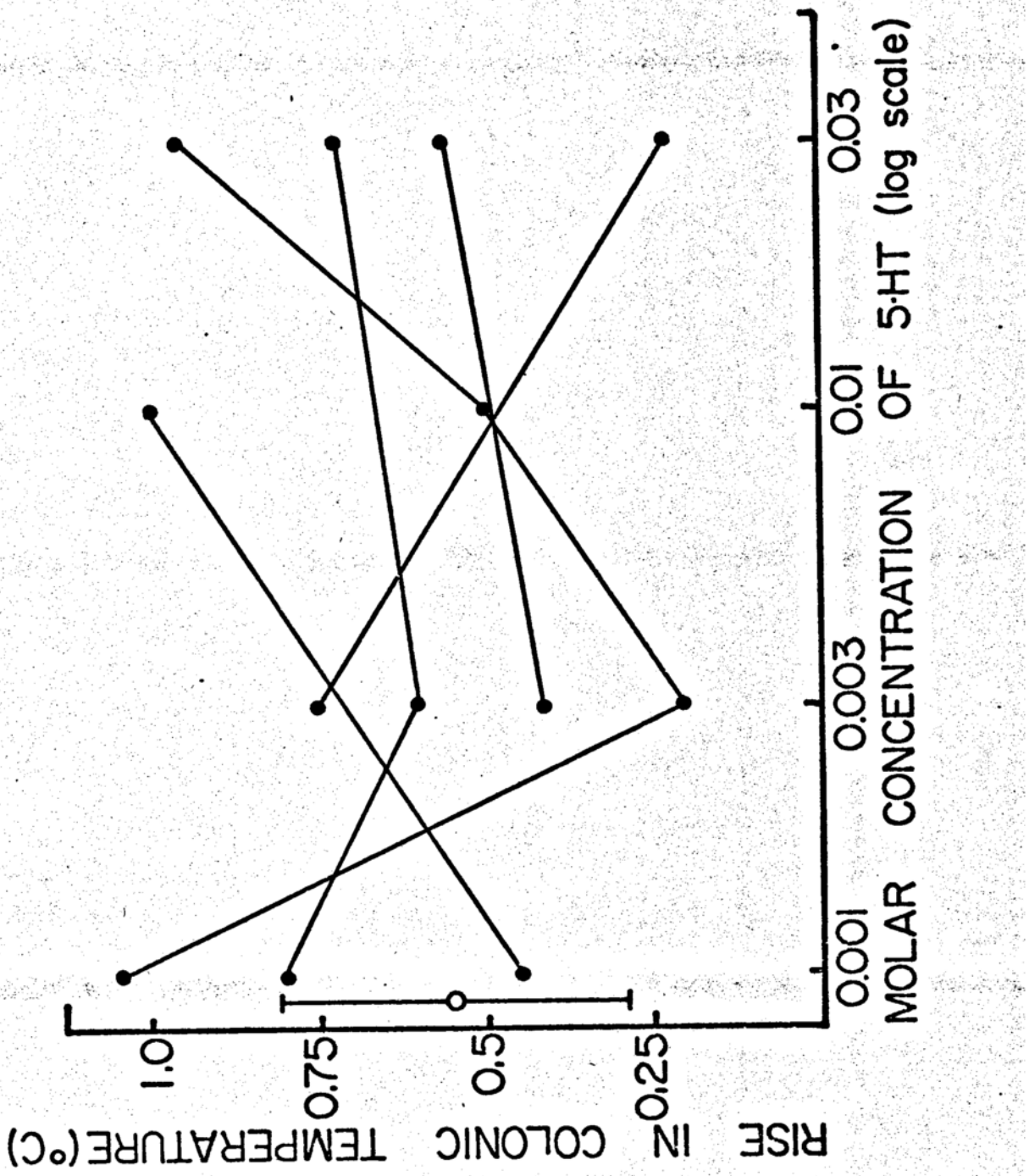

RISE IN COLONIC TEMPERATURE (°C)
1.0
0.75
0.5
0.25
0.001
0.003
0.01
0.03
MOLAR CONCENTRATION OF 5-HT (log scale)

TABLE 5

The fall and the delayed hyperthermia produced by 5-HT or control solutions.

Treatment	n[c]	INITIAL FALL: Maximum ΔT_c (°C) Mean ± S.E.M.	INITIAL FALL: Comparison of means[b]	FOLLOWING RISE[a]: Maximum ΔT_c (°C) Mean ± S.E.M.	FOLLOWING RISE[a]: Comparison of means[b]
0.015 M CS[d]	5	0.07 ± 0.04	P < 0.01	0.44 ± 0.13	P > 0.1
0.01 M 5-HT		0.42 ± 0.04		0.67 ± 0.36	
0.046 M CS or ACSF[e]	5	0.03 ± 0.02	P < 0.01	0.66 ± 0.28	P > 0.1
0.03 M 5-HT		0.82 ± 0.13		0.49 ± 0.12	

[a]Mean maximum increase in colonic temperature after the initial hypothermia and within 4.5 hours after an injection of 5-HT.

[b]A paired-sample Student's t-test was used to determine the significance of differences between means connected by solid lines.

[c]Number of different microinjection sites tested with each set of treatments.

[d]Creatinine sulfate.

[e]An injection of 0.046 M CS was injected at one site and ACSF at the other four sites.

G. Antagonism of the fall and the delayed rise in body temperature evoked by central injections of 5-HT.

To examine the role of 5-HT receptors in the fall and subsequent rise in body temperature evoked by 5-HT, four cats were injected intraperitoneally with methysergide, 2 mg/kg, two hours prior to an i.c. injection of 0.01 M 5-HT. Figure 13 shows the effect of methysergide on the 5-HT induced fall and the delayed hyperthermia in one of the cats. As shown in Table 6, an intraperitoneal injection of methysergide significantly reduced the fall in temperature elicited by 5-HT in the four animals. With respect to the delayed rise produced by 5-HT, although treatments #1 and #2 differ significantly, the hyperthermia was not considered to be potentiated by an intraperitoneal injection of methysergice because it was not significantly different from the hyperthermia evoked by the subsequent control injection of 5-HT.

In order to ascertain whether the antagonism of the 5-HT-induced fall in colonic temperature by a peripheral injection of methysergide was due to blockade of central 5-HT receptors, methysergide was injected into i.c. loci prior to 5-HT. At one microinjection site in each of three cats in which an i.c. injection of 0.01 M 5-HT evoked a fall in colonic temperature and a delayed hyperthermia, 0.01 M methysergide was injected 15 minutes before 5-HT. The results are summarized in Table 7. Methysergide significantly reduced the fall in body temperature but had no effect

Figure 13. The effect of pretreatment with an intraperitoneal injection of methysergide on the fall and the delayed hyperthermia evoked by an i.c. injection of 5-HT. The treatments at the microinjection site were administered in the order in which they appear in the figure legend. Agents were injected intraperitoneally (i.p.) or i.c. at the arrows. The vehicle for methysergide was 5% PEG 400. The injection of 0.015 molar CS was used as a vehicle control for 5-HT.

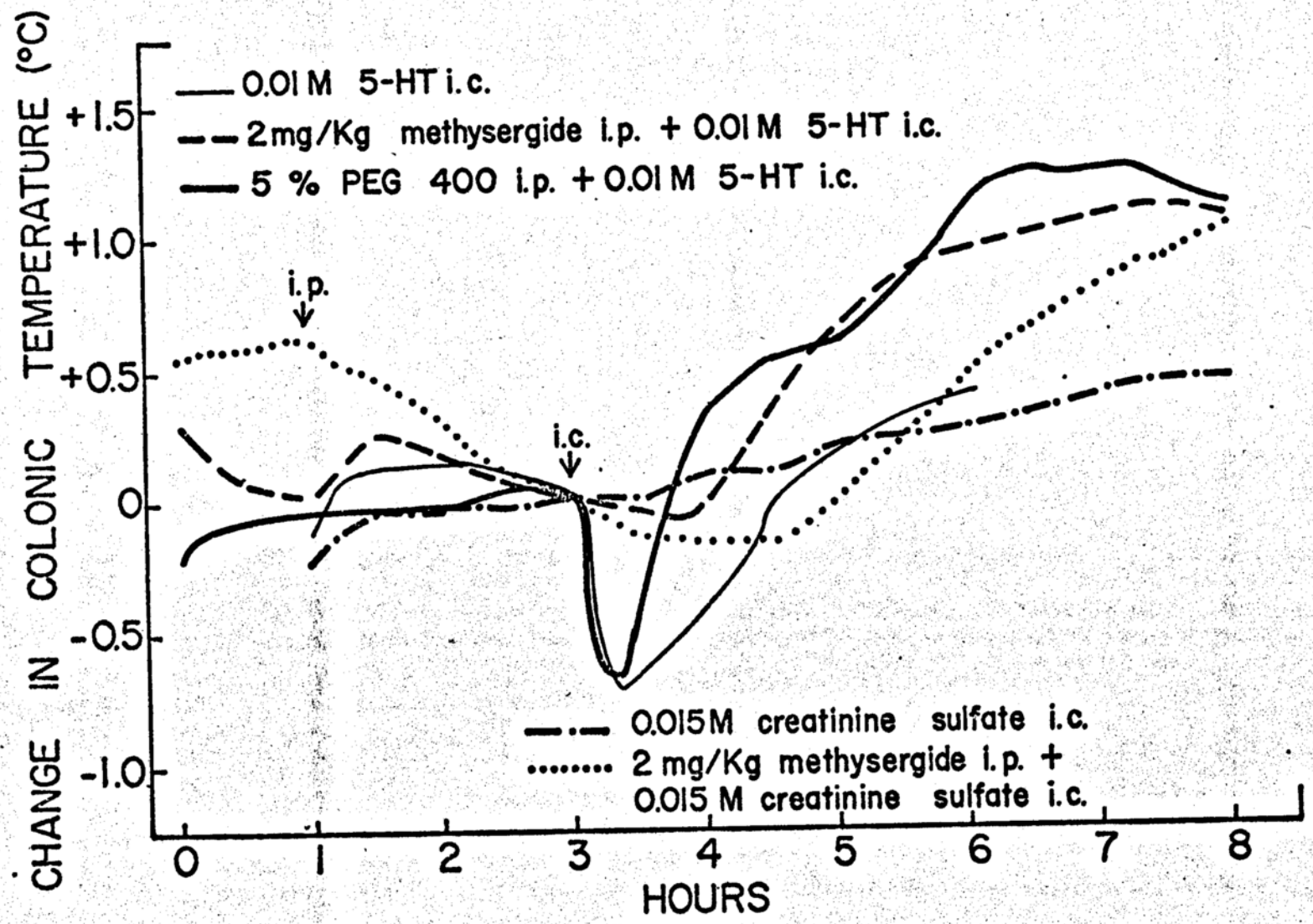

0.01 M 5-HT i.c.
2mg/Kg methysergide i.p. + 0.01 M 5-HT i.c.
5 % PEG 400 i.p. + 0.01 M 5-HT i.c.
i.p.
i.c.
0.015M creatinine sulfate i.c.
2 mg/Kg methysergide i.p. +
0.015 M creatinine sulfate i.c.
CHANGE IN COLONIC TEMPERATURE (°C)
+1.5
+1.0
+0.5
0
-0.5
-1.0
0
1
2
3
4
5
6
7
8
HOURS

TABLE 6

Effect of an intraperitoneal injection of methysergide on the fall and the delayed rise in colonic temperature elicited by an intracerebral injection of 5-HT.[a]

INITIAL FALL[b]

CS[c]	2	3	1
	methysergide+5-HT	5%PEG400+5-HT	5-HT
0.00±0.00	0.08±0.03	or	0.53±0.07
		saline+5-HT	
		0.41±0.12	

DELAYED RISE[d]

1		3	2
5-HT	CS	5%PEG400+5-HT	methysergide+5-HT
0.31±0.15	0.46±0.07	or	0.68±0.21
		saline+5-HT	
		0.55±0.23	

The data presented represent the responses to an i.c. injection of 0.01 M 5-HT or 0.015 M CS in one locus in each of four cats. Methysergide, 2 mg/kg, or an equivalent volume of normal saline was injected intraperitoneally two hours prior to an i.c. injection of 0.01 M 5-HT.

[a] Each cat received the entire series of treatments. The numbers above the treatments represent the order in which the treatments were administered. The position of the ACSF or CS injections in the treatment sequence varied among animals and is therefore not assigned a treatment number. Any two means not underscored by the same line are significant different ($p < 0.05$).

[b] Mean maximum increase in colonic temperature (°C)±S.E.M. occurring during the first rise in colonic temperature after an injection of 5-HT or during the same time period after an injection of CS.

[c] Creatinine sulfate.

[d] Mean maximum increase in colonic temperature (°C)±S.E.M. occurring either after the first rise in colonic temperature and within 4.5 hours after an injection of 5-HT or within 4.5 hours after an injection of CS.

TABLE 7

Effect of a preceding intracerebral injection of methysergide on the fall and the delayed rise in colonic temperature elicited by an intracerebral injection of 5-HT.[a]

INITIAL FALL[b]

4 methysergide +CS	2 methysergide +5-HT	CS[c]	5 5-HT	1 5-HT	3 5-HT
0.00±0.00	0.04±0.02	0.11±0.06	0.21±0.09	0.40±0.05	0.46±0.12

DELAYED RISE[d]

1 5-HT	CS	2 methysergide +5-HT	3 5-HT	5 5-HT	4 methysergide +CS
0.25±0.10	0.39±0.22	0.60±0.04	0.64±0.32	0.70±0.13	0.93±0.18

The data presented represent responses to an i.c. injection of 0.01 M 5-HT or 0.015 M CS in one locus in each of three cats. An i.c. injection of 0.01 M methysergide was administered into the injection site fifteen minutes prior to an injection of 0.01 M 5-HT or 0.015 M CS.

a,b, c and d
As in Table 6

on the delayed hyperthermia. An example of the effect of methysergide pretreatment in one of the three cats is shown in Figure 14.

H. Cholinergic pathway mediating hypothermia and the delayed hyperthermia.

In preliminary experiments, the possible involvement of a muscarinic cholinergic pathway in mediating the hypothermia and the delayed rise in body temperature elicited by an i.c. injection of 5-HT was examined using peripheral injections of atropine sulfate. Two cats in which an i.c. injection of 5-HT evoked a fall in colonic temperature and a delayed hyperthermia were pretreated with atropine sulfate, 2 mg/kg, i.p., 90 minutes prior to an i.c. injection of 5-HT. The fall in body temperature produced by 0.01 M 5-HT in one cat was potentiated, whereas in the other cat it was prevented by the atropine sulfate pretreatment. The delayed rise in body temperature produced by 5-HT was not affected by the atropine sulfate pretreatment.

Although a peripheral injection of atropine sulfate did not affect the fall in a consistent manner, 0.02 M l-hyoscyamine injected into microinjection sites 20 minutes prior to the 5-HT injection appeared to antagonize the initial fall in body temperature elicited by 5-HT. Figures 15 and 16 show the effect of an i.c. injection of l-hyoscyamine on the fall and the delayed rise in body temperature evoked by 5-HT.

Figure 14. The effect of pretreatment with an i.c. injection of methysergide on the fall and delayed hyperthermia produced by an injection of 5-HT. The injection of methysergide was administered at the first arrow 15 minutes before injecting 5-HT or CS into the site (second arrow). Creatinine sulfate and 5-HT were only injected i.c. at the second arrow. The treatments were administered in the order in which they appear in the figure legend. The injection of 0.015 molar CS was used as a vehicle control for 5-HT.

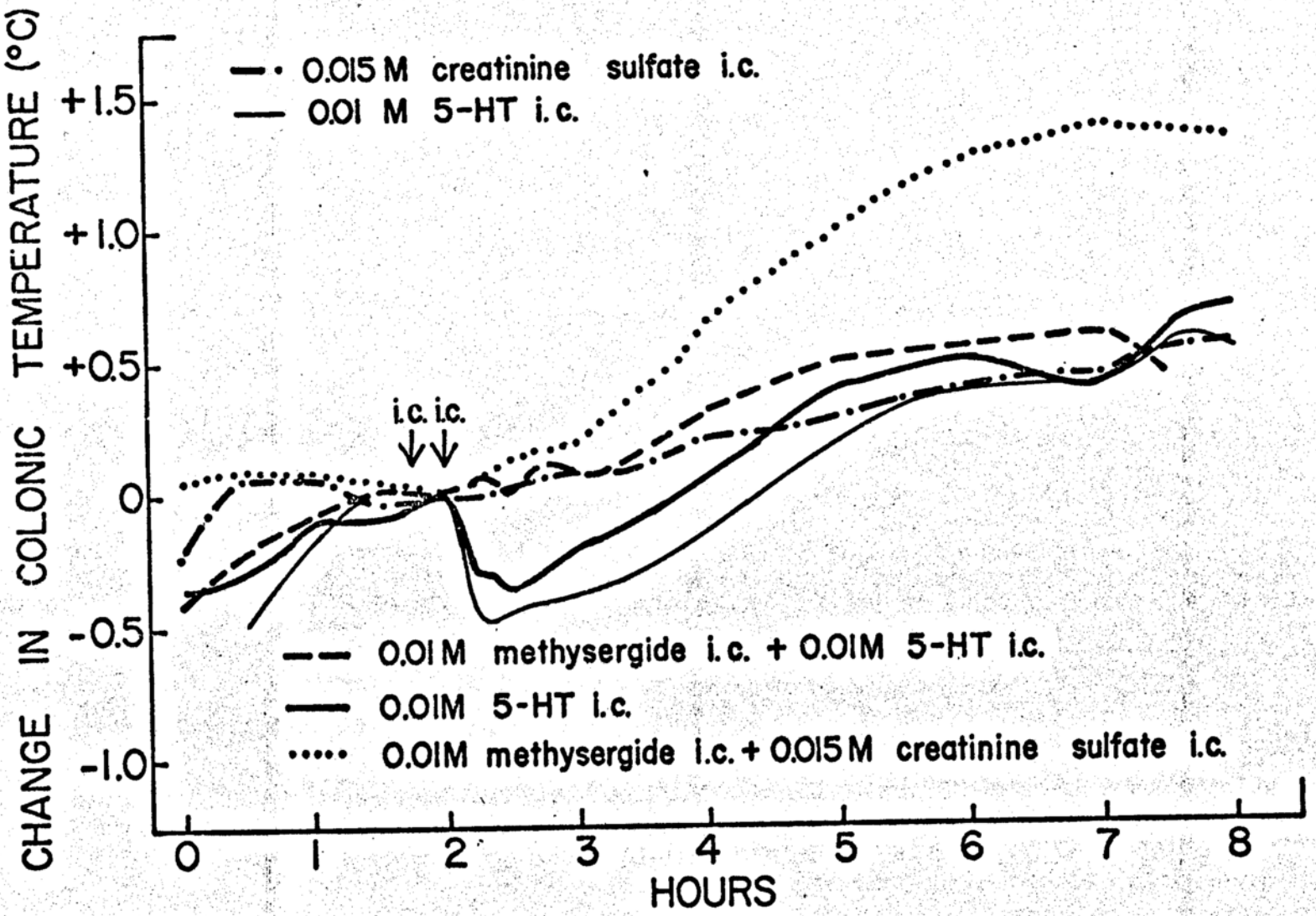

CHANGE IN COLONIC TEMPERATURE (°C)
+1.5
+1.0
+0.5
0
-0.5
-1.0
0.015 M creatinine sulfate i.c.
0.01 M 5-HT i.c.
i.c. i.c.
0.01 M methysergide i.c. + 0.01M 5-HT i.c.
0.01M 5-HT i.c.
0.01M methysergide i.c. + 0.015 M creatinine sulfate i.c.
0
1
2
3
4
5
6
7
8
HOURS

Figure 15. The effect of pretreatment with an i.c. injection of l-hyoscyamine on the fall and the delayed hyperthermia initiated by an injection of 5-HT. The injection of l-hyoscyamine was administered at the first arrow 20 minutes before injecting CS or 5-HT into the site. Creatinine sulfate and 5-HT were only injected i.c. at the second arrow. The treatments were administered in the order in which they appear in the figure legend. The injection of 0.015 molar CS was used as a vehicle control for 5-HT.

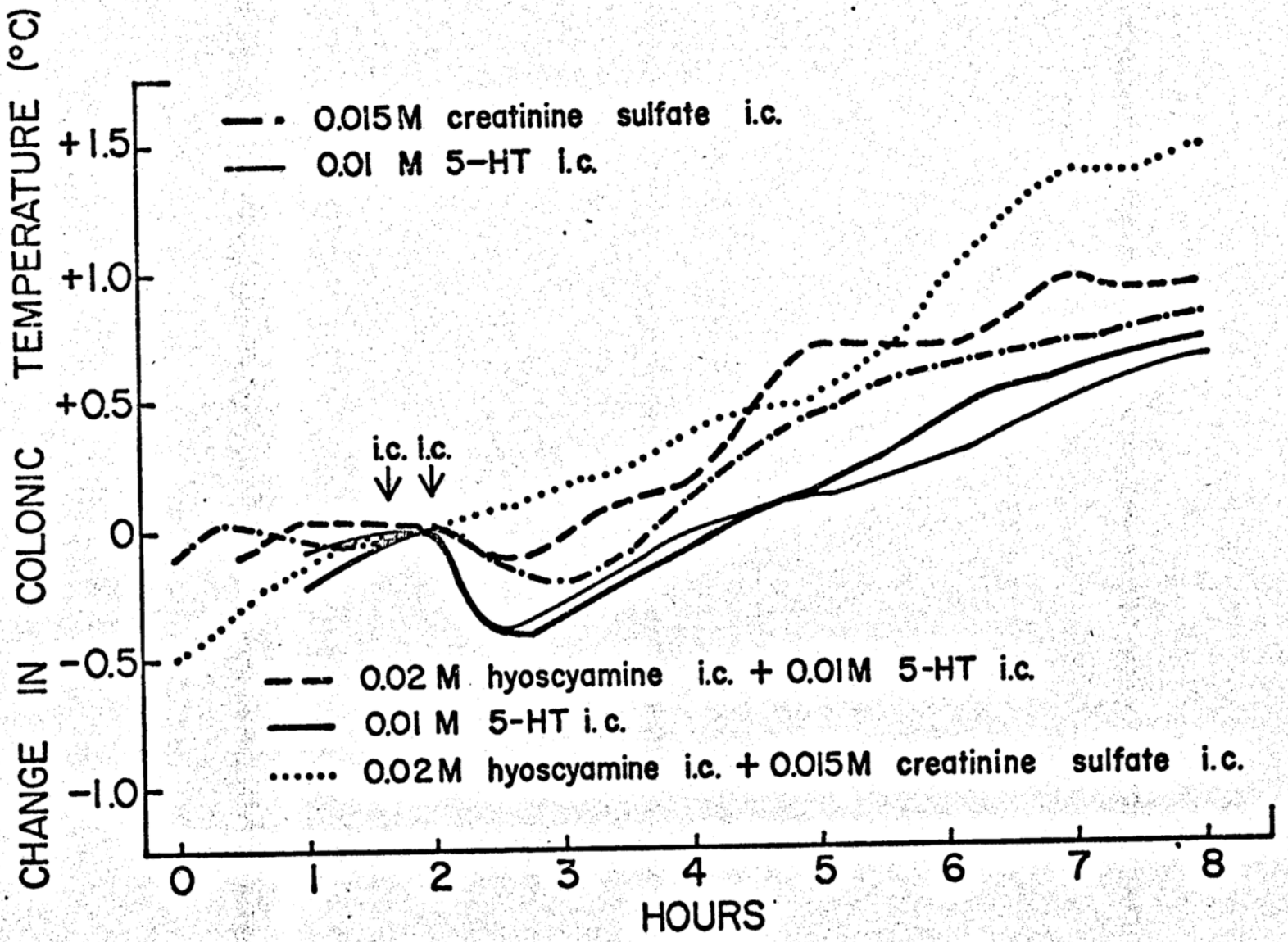
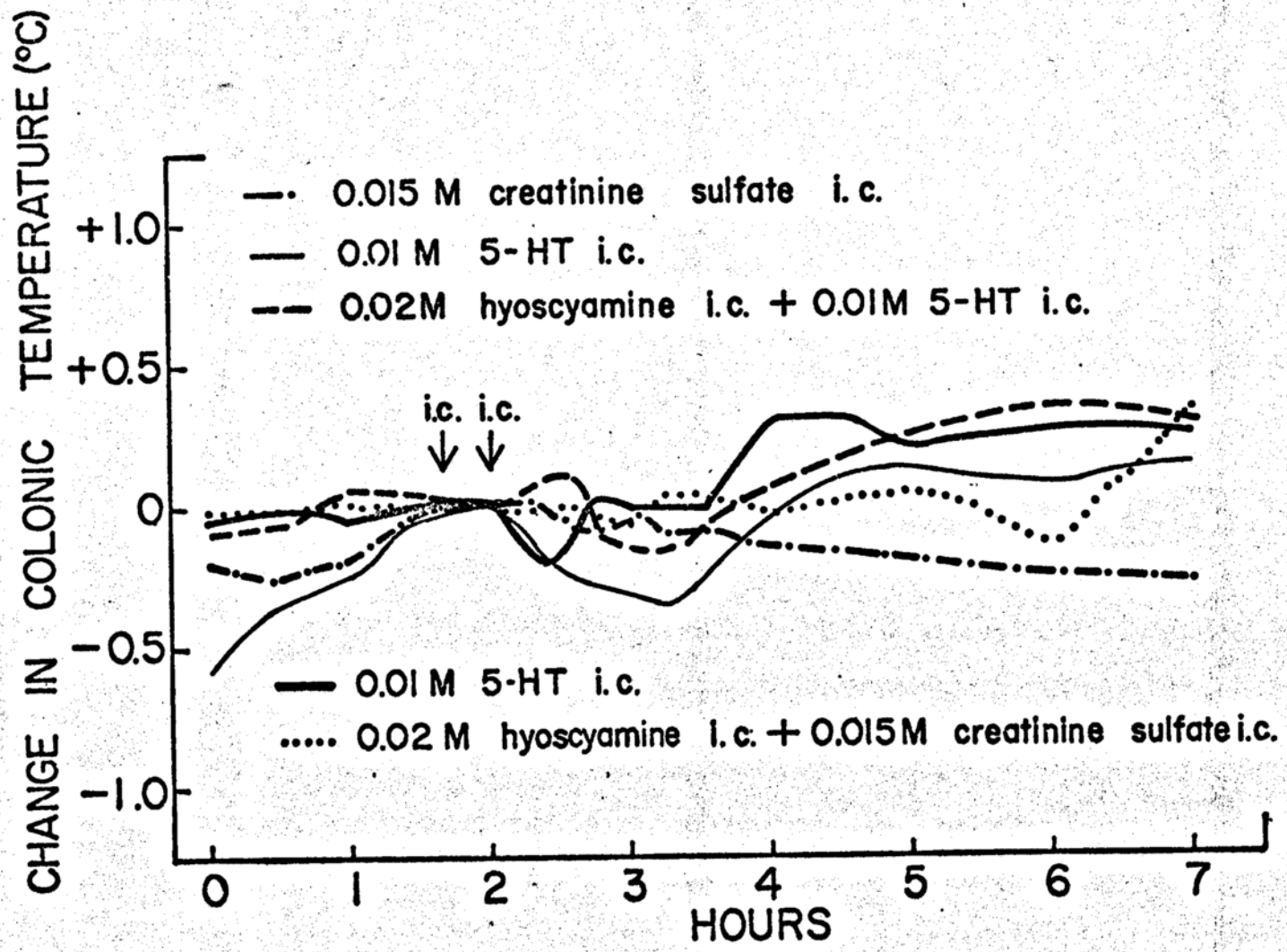

CHANGE IN COLONIC TEMPERATURE (°C)
+1.5
+1.0
+0.5
0
-0.5
-1.0
0.015 M creatinine sulfate i.c.
0.01 M 5-HT i.c.
i.c. i.c.
0.02 M hyoscyamine i.c. + 0.01M 5-HT i.c.
0.01 M 5-HT i.c.
0.02 M hyoscyamine i.c. + 0.015M creatinine sulfate i.c.
0
1
2
3
4
5
6
7
8
HOURS

Figure 16. The effect of pretreatment with an i.c. injection of l-hyoscyamine on the fall and subsequent hyperthermia initiated by an injection of 5-HT in the preoptic area of the hypothalamus. The injection of l-hyoscyamine was administered at the first arrow 20 minutes before injecting CS or 5-HT into the site. Creatinine sulfate and 5-HT were only injected i.c. at the second arrow. The treatments were administered in the order in which they appear in the figure legend. The injection of 0.015 molar CS was used as a vehicle control of 5-HT.


CHANGE IN COLONIC TEMPERATURE (°C)
+1.0
+0.5
0
−0.5
−1.0
0.015 M creatinine sulfate i. c.
0.01 M 5-HT i.c.
0.02M hyoscyamine i.c. + 0.01M 5-HT i.c.
i.c. i.c.
0.01 M 5-HT i.c.
0.02 M hyoscyamine I. c. + 0.015M creatinine sulfate i.c.
0
1
2
3
4
5
6
7
HOURS

I. Diencephalic loci responsive to intracerebral injections of 5-HT.

The regions in the diencephalon where i.c. injections of 0.01 or 0.03 M 5-HT evoked an initial hyperthermia are shown in Figure 17. The data confirm the finding of Feldberg and Myers (1965) that 5-HT elicits a hyperthermia when injected into the anterior hypothalamus. However, some sites outside the anterior hypothalamus were also responsive. Injections of 5-HT into 19 sites in the area bounded by the stereotaxic coordinates AP13.0 to AP10.5, L0.0 to 4.0, H4.0 to -5.0 produced a biphasic hyperthermia. The specific areas in the diencephalon where i.c. injections of 5-HT evoke this response include the anterior hypothalamic area and the third ventricle in the coronal planes AP13 to AP12. In addition, single responsive sites were found in the nucleus reuniens, the reticular nucleus of the thalamic and regions around the mammillothalamic tract and fornix in the tuberal hypothalamus.

The areas of the diencephalon where 5-HT elicited a hypothermia are illustrated in Figure 18. Injections of 5-HT into 26 sites in the area bounded by the stereotaxic coordinates AP16.5 to AP13.5, L0.5 to 4.0, H2.0 to -5.5 produced this response. The specific areas in the diencephalon where i.c. injections of 5-HT elicit a hypothermia include the preoptic area, ventral septal area, ventral part of the fornix and the diagonal band of Broca.

Figure 19 summarizes the data from the 15 cats, each of

Figure 17. Serial frontal sections of the cat brain illustrating the approximate locations of sites at which injections of 0.01 or 0.03 M 5-HT elicited a reproducible initial rise in body temperature over 0.25° C (solid circles) or over 0.5° C (stars) within 30 minutes of the injection. Open circles which enclose a small solid circle represent sites at which 5-HT produced an immediate rise in body temperature which could not be replicated. Each open circle represents a site at which 5-HT failed to elicit an initial rise in body temperature of at least 0.25° C within 30 minutes of the injection. Six additional negative sites in the mesencephalon are not illustrated: (AP6.0, L0.5, H1.0; AP6.0, L0.5, H0.0; AP6.0, L0.5, H-1.0; AP6.0, L2.5, H1.0; AP6.0, L2.5, H0.0; AP6.0, L2.5, H-1.0). AC, anterior commissure; AH, anterior hypothalamic area; CAU, caudate nucleus; CC, corpus callosum; DB, diagonal band of Broca; EN, endopeduncular nucleus; F, fornix; GP, globus pallidus; IC, internal capsule; M, mammillothalamic tract; MD, medial dorsal thalamic nucleus; NR, reticular thalamic nucleus; OC, optic chiasm; OT, optic tract; PH, posterior hypothalamic area; PO, preoptic area; PU, putamen; RE, nucleus reuniens; S, septum; VA, ventral anterior thalamic nucleus; VM, ventromedial hypothalamic nucleus.

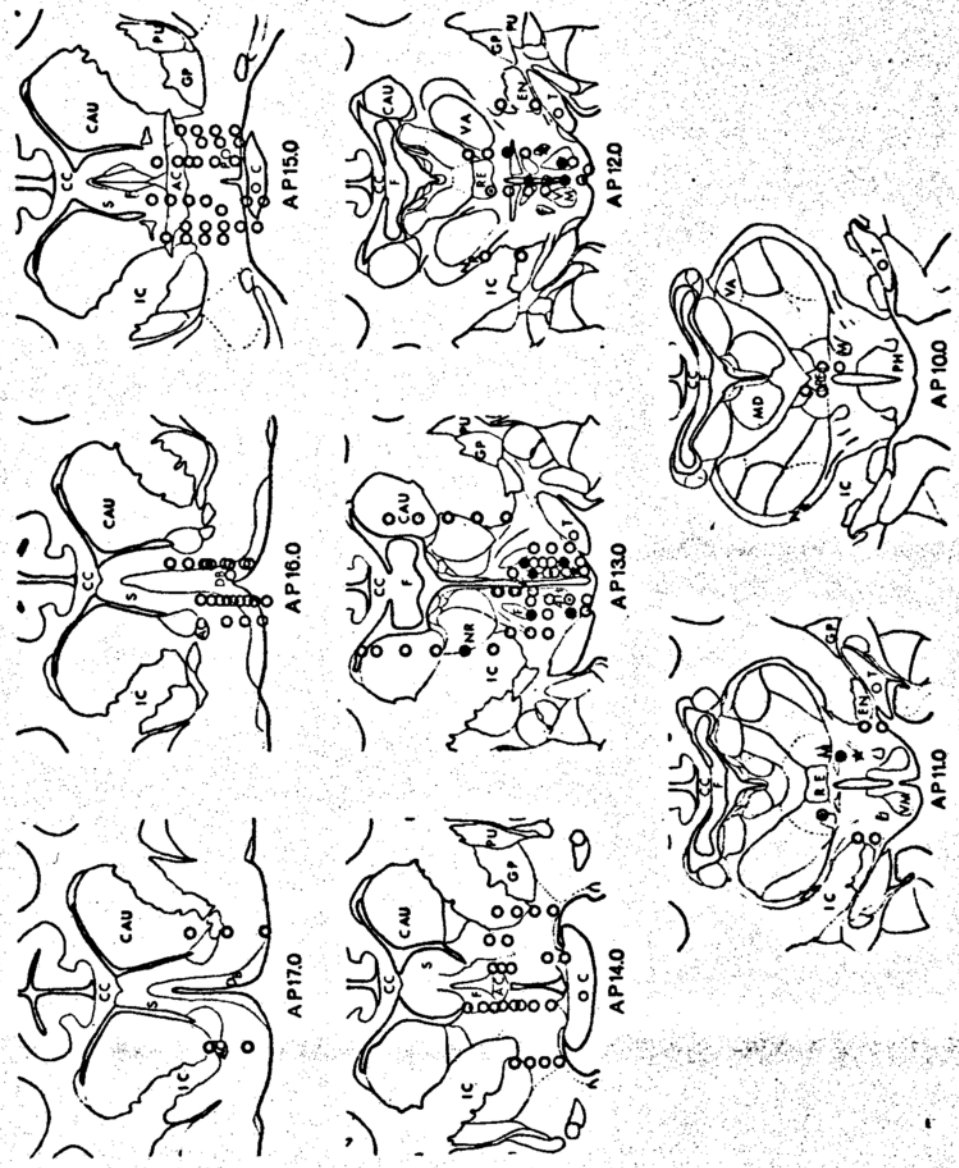

AP 15.0
AP 12.0
AP 10.0
AP 16.0
AP 13.0
AP 11.0
AP 17.0
AP 14.0
CAU
GP
PU
IC
CC
S
VA
MD
EN
RE
PN
NR
SN

Figure 18. Serial frontal sections of cat brain illustrating the approximate locations of sites at which injections of 0.01 or 0.03 M 5-HT elicited a hypothermia over 0.25° C (solid circles) or 0.5° C (stars) within 30 minutes of the injection. Each open circle represents a site at which 5-HT failed to elicit a hypothermia of at least 0.25° C within 30 minutes of the injection. Six additional negative sites in the mesencephalon are not illustrated: (AP6.0, L0.5, H1.0; AP6.0, L0.5, H0.0; AP6.0, L0.5, H-1.0; AP6.0, L2.5, H1.0; AP6.0, L2.5, H0.0; AP6.0, L2.5, H-1.0). AC, anterior commissure; AH, anterior hypothalamic area; CAU, caudate nucleus; CC, corpus callosum; DB, diagonal band of Broca; EN, endopeduncular nucleus; F, fornix; GP, globus pallidus; IC, internal capsule; M, mammillothalamic tract; MD, medial dorsal thalamic nucleus; NR, reticular thalamic nucleus; OC, optic chiasm; OT, optic tract; PH, posterior hypothalamic area; PO, preoptic area; PU, putamen; RE, nucleus reuniens; S, septum; VA, ventral anterior thalamic nucleus; VM, ventromedial hypothalamic nucleus.

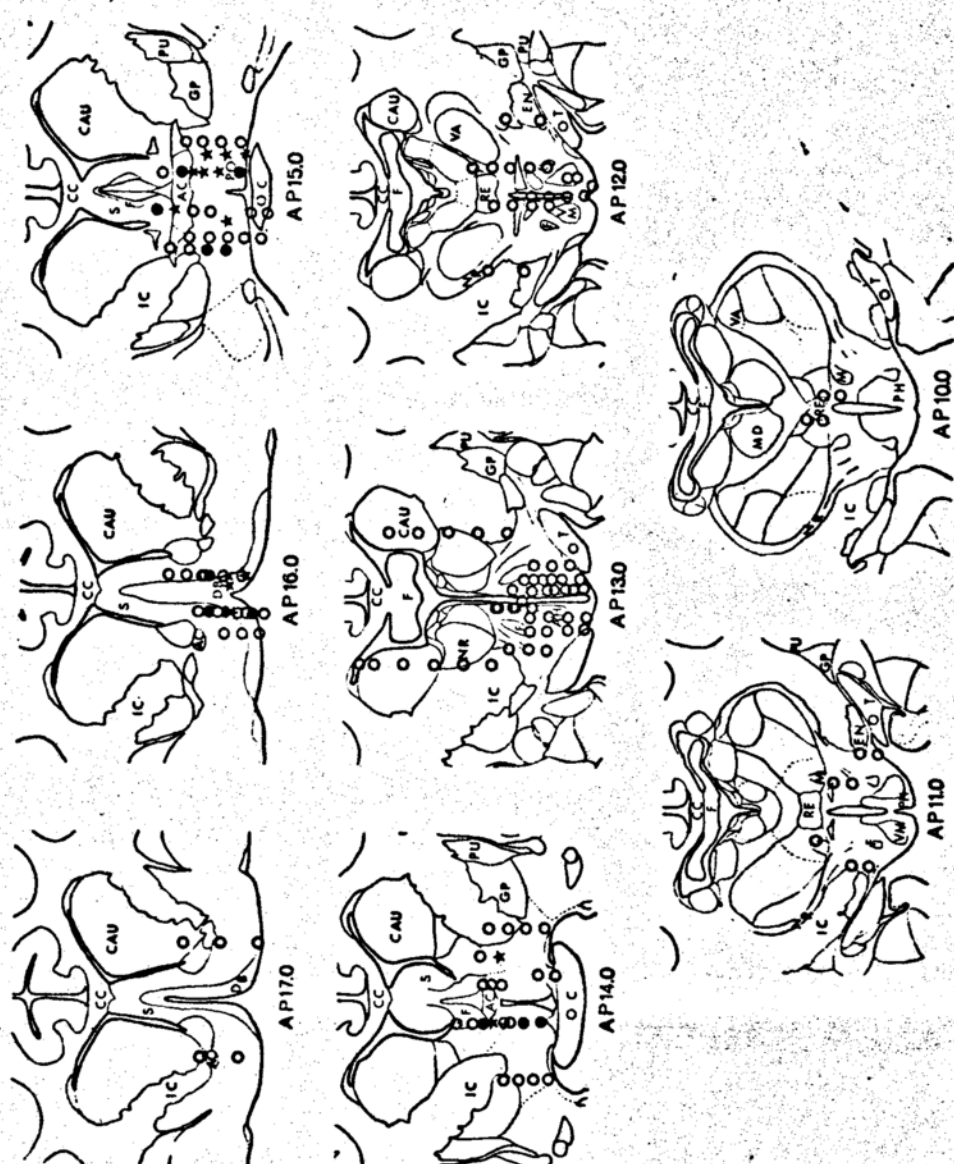
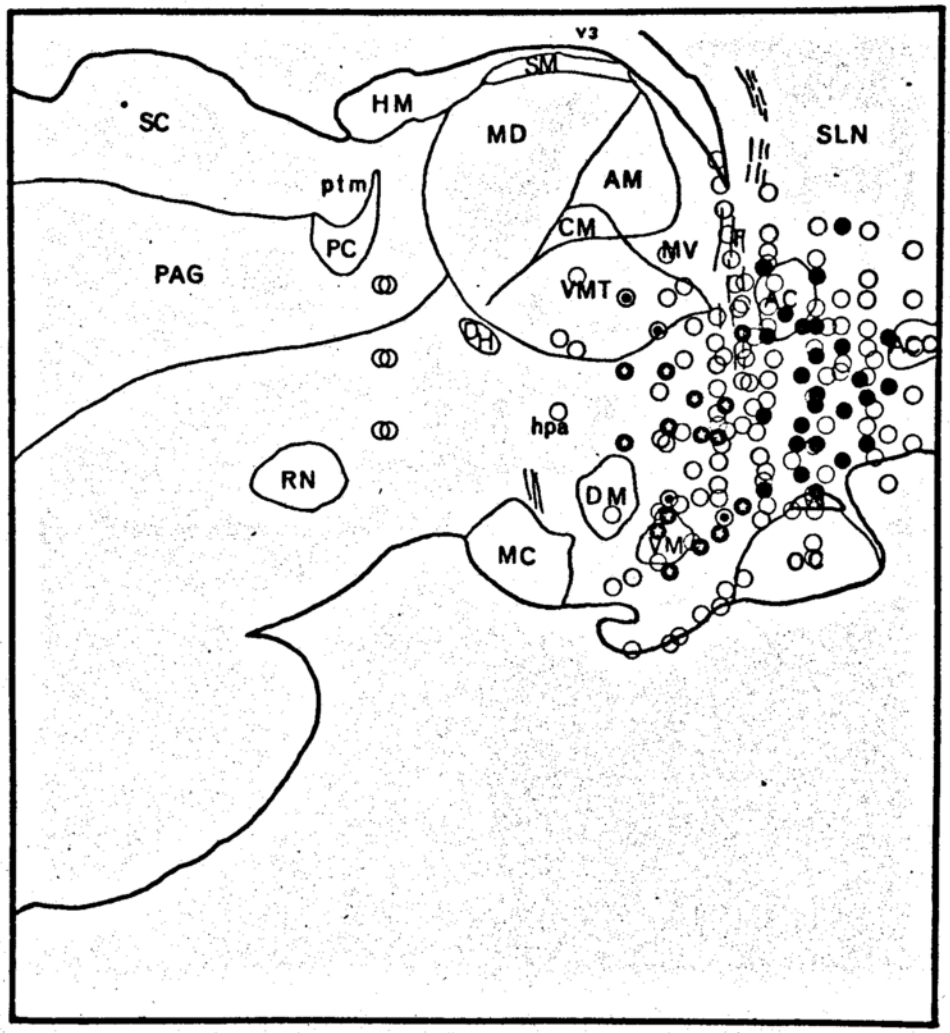

AP15.0
AP16.0
AP17.0
AP12.0
AP13.0
AP14.0
AP10.0
AP11.0
CAU
CC
IC
GP
PU
VA
MD
RE
OC
OT

Figure 19. Parasagittal projection of the cat brain illustrating the approximate locations of sites stimulated with 5-HT and the temperature responses produced. Each site was tested with 0.01 or 0.03 M 5-HT. Injection sites are projected on a parasagittal section 1.2 mm lateral to the midline. Each solid circle represents a site at which 5-HT produced a significant fall in rectal temperature within 30 minutes of the injection. Each solid circle enclosing an open star represents a site at which 5-HT produced significant hyperthermia within 30 minutes of the injection. Each open circle enclosing a small solid circle represents a site at which 5-HT produced a hyperthermia within 30 minutes of the injection which could not be replicated. Each open circle represents a site at which 5-HT failed to elicit an initial hyperthermia or hypothermia of at least 0.25^{0} C within 30 minutes of the injection. AC, anterior commissure; ACC, nucleus accumbens; AM, anteromedial nucleus; CM, central medial nucleus; DH, dorsal hypothalamic nucleus; DM, dorsomedial hypothalamic nucleus; F, fornix; HM, medial habenular nucleus; hpa, posterior hypothalamic area; MC, mammillary complex; MD, mediodorsal thalamic nucleus; MV, medioventral nucleus; OC, optic chiasm; PAG, periaquaductal grey; PC, posterior commissure; ptm, medial pretectal area; RN, red nucleus; SC, superior colliculus; SLN, lateral septal nucleus; SM, stria medullaris thalami; V3, third ventricle; VM, ventromedial hypothalamic nucleus; VMT, ventromedial thalamic nucleus.


v3
SM
HM
SC
MD
SLN
AM
ptm
CM
PC
MV
PAG
VMT
hpa
RN
DM
VM
MC

which received an i.c. injection of 0.01 or 0.03 M 5-HT, at one to thirty-three different loci. All the injection sites are represented on a single parasagittal section 1.2 mm lateral to the midline (Berman, 1968).

IV. DISCUSSION

A. The biphasic hyperthermia elicited by 5-HT.

The dose-related nature of ΔT_c and 50% $\Delta T_c/\Delta T$ for the first phase of the hyperthermia evoked by 5-HT suggests the possibility that specific pharmacological receptors are involved in mediating this response. Dose response relations with respect to temperature have been used by other investigators as indicative that specific pharmacological receptors may be involved in mediating the response (Beckman, 1970; Bruinvels, 1970; Rudy and Wolf, 1971). In addition, the ability of methysergide to reduce the first phase suggests that 5-HT receptors are involved in the response. However, 5-HT receptors are unlikely to be involved in the second phase of the hyperthermia since ΔT_c for this phase did not increase in a dose-dependent manner.

Although pharmacological receptors may be mediating the initial rise in temperature, the possible involvement of other mechanisms and receptors in the response was also investigated. Only i.c. injections of serotonin, cholinomimetic substances, prostaglandins and pyrogens have been shown to produce an immediate rise in body temperature in the cat. The hyperthermia initiated by intrahypothalamic injections of cholinergic agonists is probably mediated by muscarinic receptors (Blanton et al., 1974). However, muscarinic receptors are probably not involved in mediating the first phase of the hyperthermia elicited by 5-HT, because

i.c. injections of l-hyoscyamine, at doses higher than those which antagonized the hyperthermia initiated by the cholinergic agonist, carbamylcholine (Blanton et al., 1974), failed to antagonize the first phase of the rise in temperature evoked by 5-HT. Prostaglandins appear to mediate the fever produced by pyrogen; inhibition of the de novo synthesis of prostaglandins by indomethacin and acetaminophen has been shown to significantly reduce both the hyperthermia and the prostaglandin release evoked by intracranial injections of pyrogen (Feldberg and Gupta, 1973; Feldberg et al., 1973). The first phase of the hyperthermia induced by 5-HT, therefore, is probably not dependent upon an action of released prostaglandins since indomethacin failed to alter this response.

There are several possible explanations for the failure of peripheral injections of cinanserin, SQ10,631 and metergoline to antagonize the first phase of the hyperthermia evoked by 5-HT. For example, the doses and pretreatment times in the preliminary experiments may not have been appropriate for optimal expression of the activity of these antagonists. Another possible explanation is that the 5-HT receptors mediating the first phase of the biphasic hyperthermia may not be affected by these 5-HT antagonists. This latter explanation is supported by the microiontophoretic studies indicating the possible existence of two types of 5-HT receptors in the CNS (Segal and Bloom, 1974; Haigler and Aghajanian, 1974b). Therefore a situation similar to

the proposed existence of two types of 5-HT receptors in the guinea pig ileum (Gaddum and Picarelli, 1957) may exist in the CNS. Finally, in the case of metergoline, one must consider the possibility that a slowly formed metabolite is the active compound (Baldrati et al., 1965).

The ability of indomethacin to abolish the second peak of the hyperthermic response strongly suggests that this peak is dependent upon released prostaglandins for its mediation. On the other hand, the lack of an effect of an intraperitoneal injection of methysergide on the second peak cannot be offered as proof that 5-HT receptors are not involved in the genesis of this phase of the response. Whereas the action of indomethacin usually persists for 10 or more hours, that of peripherally injected methysergide lasts only an hour or two into the period when the secondary phase would be expected to develop. In this connection, a portion of the apparent second phase of the hyperthermia which is illustrated in Figure 7 is actually only a partial recovery from the hypothermic effects of methysergide. The figures appearing in Table 2 are not similarly influenced, for on the basis of control experiments in which methysergide alone was administered intraperitoneally, that portion of the second phase which is a consequence of recovery from methysergide has been substracted from the observed hyperthermia. It should also be clear from the present data that neither an initial hyperthermia nor 5-HT receptor activation during the first one or two hours after a 5-HT injection is required

for the development of the second phase.

In contrast to the lack of effect of peripheral injections of methysergide, central injections of this antagonist potentiated the second phase of the hyperthermia. Although the potentiation may be due to an increased traumatization of the injection site, since the site was injected twice in one experimental session. It seems unlikely that this is due to an increased traumatization of the injection site because the hyperthermia produced by creatinine sulfate was not similarly potentiated. It therefore seems possible that blockade of 5-HT receptors and/or blockade of neuronal uptake of 5-HT by methysergide (Shaskan and Snyder, 1970) produced an augmented concentration of free 5-HT within the extracellular spaces surrounding the injection site. This excess 5-HT might then cause prostaglandin release by some non-specific action or by an action at 5-HT receptors not susceptible to blockade by methysergide. This speculative explanation assumes, of course, that intracerebrally injected methysergide persists at the injection site throughout the development of the second phase of the 5-HT-induced hyperthermia. At the present time, there is no evidence to support this assumption.

In the present experiments, 5-HT was injected directly into the diencephalon, and it was found that indomethacin reduced only the second phase, whereas acetaminophen (100 mg/kg) reduced only the first phase. Indomethacin is known to be a more potent inhibitor of brain prostaglandin synthesis

than acetaminophen (Flower and Vane, 1972; Willis et al., 1972). Indomethacin (2 mg/kg) was shown by Feldberg et al. (1973) to be as effective as acetaminophen (50 mg/kg) in preventing the fever and reducing to normal the increased levels of prostaglandins in the cerebrospinal fluid evoked by an intraventricular injection of endotoxin. Furthermore, indomethacin (2 mg/kg) was able to produce the above effects within 2 hours of the injection. Therefore, because indomethacin (10 mg/kg) administered 3 hours prior to the injection of 5-HT did not alter the first phase of the hyperthermia elicited by the indoleamine, the reduction of the first phase by acetaminophen (100 mg/kg) may represent a non-specific effect of a high dose of the latter drug. The failure of acetaminophen to reduce the second phase is probably due to the short duration of action of acetaminophen. The antipyretic effect (Milton and Wendlandt, 1968), inhibition of prostaglandin synthesis (Feldberg et al., 1973) and the plasma levels (Welch et al., 1966) of acetaminophen, 50 mg/kg i.p., usually decreased about four hours after the injection.

In contrast to the finding that acetaminophen, 50 mg/kg i.p., did not alter the first phase of the biphasic hyperthermia evoked by an i.c. injection of 5-HT, Milton and Wendlandt (1971b) found that pretreatment with acetaminophen, 50 mg/kg i.p., totally abolished the fever evoked by an injection of 5-HT into the third ventricle of the cat. Unfortunately, it is not clear from their data whether the

intraventricular injections evoked a biphasic hyperthermia. It seems possible that in their experiments insufficient 5-HT penetrated into the hypothalamus to evoke the initial, specific serotonergic response. In this event, all of the hyperthermia evoked by 5-HT could be attributable to the release of prostaglandins, and inhibition of prostaglandin synthesis by acetaminophen would be expected to abolish the effect.

In the experiments of Feldberg and Myers (1964, 1965) in which 5-HT was injected intraventricularly and intracerebrally in the cat, the first peak of the response was greatly overshadowed in both magnitude and duration by the second peak. Since the amount of 5-HT injected into the hypothalamus in their experiments and ours was approximately the same, it is difficult to explain the presence of the dominant second peak observed in their studies. However, the results of the present report indicate that it is possible to evoke a significant specific serotonergic response in the cat without the concomitant evocation of a large and persistent secondary hyperthermia. In our studies, the mean magnitude of the second peak did not differ significantly from that of the first and, in a few instances, no secondary hyperthermia was observed. Moreover, the mean magnitude of the second peak evoked by an injection of 5-HT did not differ significantly from that evoked by injections of control solutions. With regard to hyperthermia, therefore, it can be concluded that the sole specific action of 5-HT was the evocation of a

short-latency, short-duration temperature increase through the activation of methysergide-sensitive 5-HT receptors.

Even though the data supports the idea that endogenous 5-HT within the preoptic area and the hypothalamus is a neurotransmitter involved in temperature regulation, the peripheral thermoregulatory effectors mediating these responses is far from clear. Feldberg and Myers (1965) stated that at an environmental temperature of 20 to 22° C shivering, ear vasoconstriction and an increased rate of respiration occurred during the biphasic hyperthermia, although quantitative data were lacking. However, in the present experiments at an environmental temperature of 20° C, the ears were vasoconstricted prior to microinjections of 5-HT and no increased vasoconstriction was observed after the injection. Quantitative data on the other two parameters are not yet available.

As shown previously, the areas in the diencephalon at which 5-HT evoked an immediate rise in body temperature are located posterior to the coronal plane formed by the anterior commissure and optic chiasm. Although the areas of the diencephalon at which 5-HT evokes an immediate rise in body temperature are located mainly within the thermosensitive region (Eisenman and Jackson, 1967; Edinger and Eisenman, 1970; Jell, 1973), the thermosensitive region also extends anteriorly into the preoptic and ventral septal areas. In addition, in the cat a less thermosensitive zone extends caudally through the hypothalamus back to the level of the

mammillary bodies (Edinger and Eisenman, 1970). This latter zone remains to be screened for sensitivity to 5-HT. Nevertheless, the data indicate that the region within which 5-HT elicits an immediate hyperthermia in the cat is similar to the area in the monkey where the indoleamine evokes a hyperthermia. However, in the monkey, i.c. injections of 5-HT between the anterior commissure and the optic chiasm also initiated an immediate hyperthermia (Myers and Yaksh, 1969). Finally, it should be pointed out that no data is presented describing or illustrating the areas where i.c. injections of 5-HT cause a delayed hyperthermia.

B. <u>The hypothermia and the delayed rise in temperature evoked by 5-HT</u>.

With respect to the fall and subsequent rise in body temperature elicited by an injection of 5-HT, specific pharmacological receptors could be involved in the hypothermia since ΔT_c as well as 50% $\Delta T_c/\Delta T$ appear to be dose-dependent. However, it is unlikely that synaptic receptors are involved in the rise in temperature following the hypothermia since the ΔT_c did not increase in a dose-dependent manner.

In support of the idea that specific pharmacological receptors are involved in the hypothermia elicited by 5-HT is the discovery that peripheral and central injections of methysergide significantly attenuated the fall in temperature. The data obtained with atropine sulfate are inconsistent and too few animals were examined to draw any

conclusions. However, although the number of sites tested was small, i.c. injections of l-hyoscyamine appeared to antagonize the hypothermia evoked by 5-HT.

The apparent effect of l-hyoscyamine raises a number of possible interpretations of the genesis of the hypothermia. The injected 5-HT could be releasing presynaptic ACh or acting on muscarinic cholinergic receptors to initiate part of the fall in temperature. In the literature, there is no experimental evidence indicating that 5-HT can stimulate muscarinic receptors. However, in the peripheral nervous system 5-HT is known to release ACh (Harry, 1963). The possible cholinergic involvement in the hypothermia elicited by 5-HT will be discussed in a later section.

Another possible explanation of these data is that there are two different 5-HT receptors within the diencephalon involved in thermoregulation. The existence of two kinds of 5-HT receptors in the guinea-pig ileum (Gaddum and Picarelli, 1957) and in the cat superior cervical ganglion (Haefely, 1974) has been reported. In addition, as previously stated, Haigler and Aghajanian (1974b) suggested that within the CNS the steric requirements of the 5-HT receptors located on serotonergic neurons differ from those located on neurons receiving serotonergic innervation. With respect to the hypothermia evoked by the indoleamine, it is possible that 5-HT receptors are mediating the entire response and that in contrast to the 5-HT receptors mediating the initial, short-lasting hyperthermia evoked by 5-HT, l-hyoscyamine

antagonizes the stimulation of these receptors by 5-HT.

It is also possible that hyoscyamine could have antagonized the response by a non-specific blockade of 5-HT receptors. This seems unlikely, however, since this same dose of l-hyoscyamine failed to antagonize the 5-HT-induced hyperthermic effect.

It is theoretically possible that endogenous NE is mediating the hypothermia evoked by indoleamine since at the concentrations of 5-HT injected i.c. in the present study, a fairly large proportion of the indoleamine could be expected to enter noradrenergic neurons (Fuxe and Ungerstedt, 1967; Shaskan and Snyder, 1970; Iversen, 1970). If this did occur, the injected indoleamine could be releasing endogenous NE (Reid et al., 1968). The released NE could then elicit the hypothermia since Rudy and Wolf (1971) reported that i.c. injections of the amine caused a dose-dependent hypothermia by way of alpha adrenergic receptors. In support of the above possibility, the major action of 5-HT on isolated strips of cat spleen was reported to be the release of endogenous NE (Innes, 1962). The indoleamine was also reported to have a minor action on alpha adrenergic receptors on the isolated strips.

Since ergot derivatives have alpha adrenergic blocking ability (Gyermek, 1961) one can not rule out the possibility that intracerebrally injected 5-HT acts on alpha adrenergic receptors to produce the hypothermia. Furthermore since 5-HT can release presynaptic NE in the cat spleen (Innes,

1962), possibly through an action on presynaptic 5-HT receptors, the ability of methysergide to antagonize the hypothermia elicited by 5-HT in the present study does not negate the possibility that the fall in temperature is mediated by an indirect action of 5-HT. Jacobson (1967) reported that injecting a large volume and a high dose of 5-HT into the medial preoptic area produced a hypothermia accompanied by vasodilation and a reduced oxygen consumption. The present study confirms the ability of the indoleamine to produce vasodilation during the hypothermia. The relation between skin temperature and blood flow was discussed by Burton (1947). However the possible involvement of an inhibition of shivering, muscle tone, and endocrine cold defense mechanisms in the fall in temperature has not been examined.

The inability of 5-HT to provoke panting in the present study may have been due to stimulating a small area on one side of the diencephalon and the fact that the ambient temperature was maintained at 20° C. This viewpoint is supported by previous work in which heating of the hypothalamus did not elicit panting at environmental temperatures less than 29° C (Jacobson and Squires, 1970) unless a relatively large area was heated (Roberts and Robinson, 1969).

The injection sites in the diencephalon where 5-HT elicited a hypothermia of over 0.25° C within 30 minutes of the injection were located anterior to the anterior commissure and optic chiasm. Although the area of the diencephalon at which 5-HT evoked this hypothermia is within the

thermosensitive region, as pointed out earlier this thermosensitive region extends caudally into the anterior and tuberal hypothalamus where 5-HT elicits an immediate hyperthermia, and into the posterior hypothalamus an area which has not been screened for sensitivity to 5-HT. At the present time it is impossible to compare the regions in the cat where 5-HT initiates this hypothermia, with the findings in other species because other species have only been superficially examined for the ability to evoke heat loss from any specific region of the CNS.

C. Cholinergic pathway mediating the thermoregulatory effects of 5-HT.

As previously stated, evidence obtained using the monkey as an experimental subject strongly supports the idea that an efferent cholinergic pathway is mediating the hyperthermia evoked by i.c. injections of 5-HT. In the cat, atropine sulfate antagonized the initial rise in temperature produced by 5-HT, whereas peripheral injections of atropine methyl nitrate not only failed to block the rise but actually potentiated the response. Atropine methyl nitrate, because of its quaternary structure crosses the blood brain barrier poorly (Innes and Nickerson, 1970), whereas atropine sulfate enters the CNS readily. Therefore, it seems likely that there is a muscarinic cholinergic involvement in the initial rise in temperature evoked by 5-HT. However, it is improbable that a cholinergic system is mediating the second phase

of the hyperthermia since peripheral injections of atropine sulfate failed to significantly antagonize this response.

The inability of i.c. injections of l-hyoscyamine to reduce the first phase eliminates the possibility that 5-HT directly or indirectly stimulates muscarinic receptors near the site of injection. This cholinergic involvement, therefore, must be at some site distant from the injection locus. This could be in the efferent pathways which activate specific thermoregulatory effectors, in afferent pathways carrying information from non-diencephalic thermosensors or in a diencephalic thermoregulatory intergrative network.

Some type of cholinergic involvement in the fall in temperature evoked by 5-HT is suggested by the ability of i.c. injections of l-hyoscyamine to antagonize the response. As previously stated, it seems possible that the injected 5-HT could be releasing endogenous acetylcholine, either by releasing presynaptic acetylcholine or by stimulating postsynaptically the cell bodies of cholinergic interneurons whose terminals are near the site of injection. At the present time, neither alternative can be ruled out.

Additional possibilities are that the injected indoleamine stimulates 5-HT receptors which are sensitive to l-hyoscyamine or that hyoscyamine produced a non-specific blockade of 5-HT receptors. As has been mentioned previously, this latter possibility seems improbable.

It is also conceivable that several or all of the previously mentioned factors are involved in mediating the fall

in temperature elicited by 5-HT. Finally, it is unlikely that a cholinergic system is mediating the rise in temperature following the hypothermia since atropine sulfate failed to alter this response.

The possibility that a nicotinic pathway is mediating the hypothermia must also be considered. Intraventricular injections of nicotine in the cat cause a fall in body temperature (Hall, 1972; Baird and Lang, 1973). This hypothermia is probably mediated by nicotinic synapses in the posterior hypothalamus or lower brain stem, since injections of nicotines in the AH/PO area failed to cause a fall in body temperature (Blanton et al., 1974). It is therefore quite possible that a nicotinic pathway is mediating the fall in temperature evoked by i.c. injections of 5-HT.

D. Speculated role of 5-HT in thermoregulation.

In the remaining discussion it will be assumed that only the initial hyperthermia and hypothermia elicited by 5-HT represents the physiological function of the indoleamine in thermoregulation. This is assumed because 5-HT is rapidly cleared from the brain (Tozer et al., 1966; Bulat and Supek, 1967; Myers et al., 1971; Hashi et al., 1972) and because in the present study only the initial hyperthermia and hypothermia seemed to be mediated by specific serotonin receptor(s). Although 5-HT receptors may be involved in mediating the second phase, it seems more likely that this phase is related to thermoregulatory pathology. This has been

suggested (Feldberg, 1974) and indicated by experimental evidence (Bulle, 1957; Osterholm and Meyer, 1969; Osterholm and Pyensan, 1969; Welch et al., 1972). Therefore the following discussion will be in reference to only the initial hyperthermia and hypothermia initiated by 5-HT.

The general region in the diencephalon where injected 5-HT can evoke body temperature changes coincides with the area in which temperature sensitive neurons have been found. In 1938, Magoun et al. showed that heating the cat hypothalamus would activate heat loss. The AH/PO region was found to be the most sensitive, but an area less sensitive to heating extended caudally through the tuberal and posterior hypothalamus. More recent experiments substantiated this proposed distribution of thermosensitivity in the cat's diencephalon, with the exception that additional thermodetectors have been found in the ventral septal area (Eisenman and Jackson, 1967; Edinger and Eisenman, 1970). These findings raise the possibility that 5-HT may be acting on thermodetector neurons to elicit the observed body temperature changes. However, thermodetector neurons in the cat are generally not affected by 5-HT but are current-sensitive (Eisenman and Jackson, 1967; Beckman and Eisenman, 1970)[1].

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[1]Eisenman and Jackson (1967) considered a neuron to be a warm-sensitive thermodetector if its firing rate was linearly related to the local temperature and the rate of firing was more than doubled if the local temperature was increased 10^0 C ($Q_{10} > 2$). A neuron was considered to be a cold-sensitive thermodetector if its firing rate showed a linear relationship with the local temperature and the rate of firing more than doubled if the local temperature was

In contrast to the findings of Eisenman and coworkers, Jell (1973) found some thermodetectors to be sensitive to 5-HT, NE and ACh, but after comparing their responses to the above drugs with the responses of thermosensitive interneurons he reported ". . . a total lack of correlation between thermal response type and drug sensitivity." Although Jell (1973) reported that cold sensitive thermodetectors were not inhibited by 5-HT, the sample size was too small to make any conclusions. In agreement with the findings of Eisenman and coworkers, Jell (1973) reported that some thermodetectors were current-sensitive and were not affected by 5-HT, NE or ACh. Although this evidence may indicate that some thermodetectors lack synaptic receptors, Eisenman (1974) has demonstrated that stimulating the cell bodies of 5-HT and NE neurons which have synaptic terminals in the area of thermodetectors excited or depressed the thermodetectors. Based on these latter results, Eisenman (1974) suggested that it may not always be possible to adequately stimulate neural synapses using microiontophoresis or that the transmitter involved in regulating the activity of thermodetectors has not been tested. In view of the above findings, it can not be determined if microinjected 5-HT affects thermodetectors. Furthermore until the possibility is examined that

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decreased 10^0 C ($Q_{10} > 2$). Neurons with firing rates that had a non-linear relationship with the local temperature and/or had a $Q_{10} \leq 2$ were considered warm or cold-sensitive interneurons depending upon whether firing increased with warming or cooling, respectively.

thermodetector neurons in the area where 5-HT evokes a hypothermia respond differently to the indoleamine than similar neurons in the area where the initial hyperthermia is initiated by 5-HT, it probably will not be determined.

It is possible that the injected indoleamine is acting on effector pathways. Within this theoretical framework, the latency, sensitivity and magnitude of the thermoregulatory response may depend on the activation of a critical number of efferent neurons. Furthermore it is conceivable that the sensitivities of shivering, panting and vasodilation to intracerebral injections of 5-HT may differ. However, evidence for or against this latter possibility was not obtained in the present study because vasodilation was the only thermoregulatory effector system measured quantitatively.

Another possibility is that injected 5-HT may be functioning on afferent inputs to the hypothalamus. Since thermosensitive neurons may not contain 5-HT receptors (Eisenman and Jackson, 1967; Eisenman, 1974), this viewpoint would presume that there are interneurons which are excited or depressed by the injected indoleamine. The interneuron would in turn release a transmitter different from 5-HT which would affect the thermosensitive neurons. In support of the existence of these interneurons is the finding that thermodetector neurons in the AH/PO area were excited or depressed by electrically stimulating the raphé nuclei (Eisenman, 1974).

With regard to the proposed involvement of 5-HT in temperature regulation, the data from the present study suggest that there are at least two distinct regions in the diencephalon of the cat which mediate opposing temperature responses to 5-HT. Furthermore, the ability of the 5-HT receptor antagonist, methysergide, to block both the initial hyperthermia and hypothermia suggests that 5-HT receptors may be mediating both of the temperature responses.

In some species, 5-HT has been suggested to be involved in only heat loss, in others, in only heat production, and in still others, in both heat loss and heat production (Feldberg et al., 1967; Banerjee et al., 1970). This variability of the thermoregulatory effects of centrally administered 5-HT has tended to inhibit acceptance of the putative neurotransmitter function of 5-HT within the CNS systems controlling body temperature. However, it must be pointed out that in most of the studies which suggest the existence of considerable interspecies variability, 5-HT was injected intraventricularly. Unfortunately, the effect produced by an intraventricular injection will be some function (not necessarily an algebraic summation) of the effects of the injected agent on all of the 5-HT-sensitive structures which can be reached by diffusion from the ventricular CSF. The effect observed may also be dependent upon brain size; a drug present in the ventricular CSF of a large brained species must penetrate more tissue to reach deeply situated structures than in a species with a small brain. Thus,

information gained by application of the intracerebral microinjection technique is necessary to elucidate the various possible independent thermoregulatory effects of 5-HT and the sites at which these effects are mediated.

Prior to the time this investigation was initiated, the role of 5-HT in thermoregulation had been examined extensively using the intracerebral microinjection method in only three homeothermic species. The results of these studies suggested that, in two of these species, rat (Crawshaw, 1972) and rabbit (Cooper et al., 1965; Banerjee et al., 1970), 5-HT can initiate heat loss and heat gain by an action on separate regions of the brain. These findings are analogous to those of the present study, in which the cat was used. The site-dependence of the thermoregulatory effects of intracerebrally injected 5-HT in rat, rabbit and cat suggests that the apparent interspecies variability of the response to intraventricularly applied 5-HT may be more a consequence of deficiencies of the technique than of actual differences among species in the diencephalic neurochemical coding for thermoregulation. This issue can be resolved only by further microinjection mapping of the brain stem of the various species for sensitivity to 5-HT.

Of the species which could profitably be further explored by application of this method, the rhesus monkey is perhaps the most important. Some information regarding the effects of intracerebrally injected 5-HT in this species is available. Myers and Yaksh (1969) reported that low doses

of 5-HT injected into the diencephalon of the rhesus monkey evoked either no effect or hyperthermia. Based on this data and on a few early experiments in the cat (Feldberg and Myers, 1964, 1965), a neurochemical/neuroanatomical model for thermoregulation in cat and monkey has been proposed (Myers and Yaksh, 1969; Myers, 1974). This widely cited theory suggests that the sole thermoregulatory function of 5-HT within the diencephalon is as a neurotransmitter within the heat gain apparatus. However, the results of the present investigation indicate that, in the cat, 5-HT may also be involved in the mediation of heat dissipation. The Myers-Yaksh model therefore may not be applicable to this species. In addition, it must be stressed that, in the experiments of Myers and Yaksh, the preoptic, the diagonal band of Broca and ventral septal areas of the rhesus monkey were not thoroughly explored for reactivity to 5-HT. It is precisely these areas which, in the cat, mediate 5-HT-induced hypothermia. This fact and the remarkable similarity between cat and monkey with respect to the thermoregulatory consequences of intracerebral injections of putative neurotransmitters other than 5-HT (Feldberg and Myers, 1964, 1965; Myers and Yaksh, 1969; Rudy and Wolf, 1971, 1972) strongly suggests the need for further exploration of the monkey diencephalon for possible hypothermic effects of this substance.

APPENDIX 1

Composition of Artificial CSF

Constituent	g/L	moles/L	Ions	moles/L	meq/L
NaCl	7.68	0.1314	Na^+	0.1550	155.0
KCl	0.19	0.0025	K^+	0.0025	2.5
$CaCl_2$	0.14	0.0013	Ca^{++}	0.0013	2.6
$MgCl_2 \cdot 6H_2O$	0.19	0.0011	Mg^{++}	0.0011	2.2
$NaHCO_3$	1.76	0.0210	Cl^-	0.1387	138.7
Na_2HPO_4	0.18	0.0013	HCO_3^-	0.0210	21.0
Glucose	0.61	0.0034	$HPO_4^=$	0.0013	2.6
			Glucose	0.0034	

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MEDIATORS OF THE TEMPERATURE CHANGES EVOKED BY INTRACEREBRAL INJECTIONS OF 5-HT IN THE CAT

HAROLD LOUIS KOMISKEY

Under the supervision of Assistant Professor Thomas A. Rudy

The objectives of the study were to determine in the cat: (1) the involvement of 5-hydroxytryptamine (5-HT) receptors and prostaglandins in the initial and delayed increase in body temperature evoked by intracerebral (i.c.) injections of 5-HT, (2) the possibility that a specific diencephalic region(s) may mediate the hypothermia observed after certain i.c. injections of 5-HT, (3) the nature of the involvement of synaptic 5-HT receptors in the hypothermia produced by an i.c. injection of 5-HT, and (4) the potential involvement of a cholinergic pathway in mediating the body temperature changes evoked by i.c. injections of 5-HT.

Cats of either sex, were stereotaxically implanted with intracerebral guide cannulae aimed at regions within and around the hypothalamus. Two weeks following surgery, cats were partially restrained in an isolation chamber maintained at an ambient temperature of 20.0 ± 0.5^{0} C during the experimental sessions. All i.c. injections consisted of 1 µl of sterile, pyrogen-free fluid delivered at a constant rate over a 2 minute period.

Intracerebral injections of 5-HT at loci in the diencephalon located posterior to the coronal plane formed by

the anterior commissure and optic chiasm produced an initial increase in colonic temperature which was usually followed by a delayed hyperthermia. Pretreatment of the cats with the prostaglandin synthesis inhibitor, indomethacin (10 mg/kg, i.p.) antagonized the delayed rise in colonic temperature but did not alter the initial rise in body temperature elicited by i.c. injections of 5-HT. In contrast, pretreatment of the cats with the 5-HT receptor antagonist, methysergide (2 mg/kg, i.p.), or the muscarinic cholinergic antagonist, atropine sulfate (2 mg/kg, i.p.) antagonized the initial rise in colonic temperature but did not alter the delayed rise. To further characterize the type of pharmacological receptor(s) mediating the initial rise in temperature, the microinjection sites where 5-HT evoked this response were pretreated with methysergide, 0.02 M, or the muscarinic cholinergic antagonist, l-hyoscyamine, 0.02 M. The i.c. injections of methysergide antagonized the initial rise in colonic temperature produced by 5-HT, whereas the i.c. injections of l-hyoscyamine did not alter this response.

Intracerebral injections of 5-HT at microinjection sites located between the anterior commissure and optic chiasm and in telencephalic subcortical structures located anterior to the coronal plane formed by the anterior commissure and optic chiasm produced an initial hypothermia which was usually followed by a delayed rise in body temperature. Pretreatments of the cats with methysergide (2 mg/kg, i.p.) antagonized the initial fall in body temperature, but did

not alter the delayed rise in temperature. In addition, the microinjection sites where 5-HT elicited an initial hypothermia were pretreated with methysergide, 0.01 M. The methysergide pretreatment antagonized the initial fall in body temperature, but did not affect the delayed hyperthermia caused by the indoleamine.

In summary, the results obtained in this study suggest that in the cat: (1) central 5-HT receptors are involved in mediating the initial rise or fall in body temperature produced by i.c. injections of 5-HT, (2) prostaglandins are involved in mediating the delayed rise in body temperature occurring after the initial hyperthermia elicited by i.c. injections of 5-HT, (3) specific regions of the CNS mediate the initial hypothermia and regions separate from the above areas mediate the initial hyperthermia elicited by i.c. injections of the indoleamine, and (4) a muscarinic cholinergic pathway is probably mediating the initial rise in body temperature produced by i.c. injections of 5-HT.

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