THE EFFECTS ON THERMOREGULATION OF NOREPINEPHRINE BITARTRATE, 5-HYDROXYTRYPTAMINE HYDROCHLORIDE AND CARBAMYLCHOLINE CHLORIDE INJECTED INTO THE RAT SPINAL SUBARACHNOID SPACE

BY

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This thesis is dedicated to my wife Margaret whose love and understanding made the completion of this work possible and to my son Michael who helped me keep my work in perspective.

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Under the supervision of Associate Professor Thomas A. Rudy

The effects on thermoregulation of norepinephrine bitartrate (NE), 5-hydroxytryptamine hydrochloride (5-HT), and carbamylcholine chloride (CCh) injected into the lumbar spinal subarachnoid space via a chronic indwelling catheter were examined in the rat.

Intrathecal injections of NE (0.01-0.30 µmoles) and clonidine (0.0175-0.070 µmoles) produced a dose-dependent hypothermia associated with a decrease in tail skin vasomotor tone. Shivering activity was not decreased by intrathecal NE, and a transient hyperthermia preceded the hypothermia induced by intrathecal injection of the 0.30 µmole dose of NE. Intrathecal 5-HT (0.03-0.90 µmoles) elicited a dose-dependent hyperthermia accompanied by increased tail skin vasomotor tone and increased shivering. CCh injected intrathecally (0.001-0.06 µmoles) evoked a dose-dependent hyperthermia. During the period when core temperature (T_c) was rising, tail skin vasomotor tone increased and shivering-like activity was present. Once the maximum T_c

had been reached, tail skin vasodilation occurred. Vasodilation persisted until T_c had returned to normal.

Intravenous injections of 5-HT (0.30 and 0.90 µmoles) or CCh (0.006 and 0.03 µmoles) caused no thermoregulatory effect. The effects of these agents injected intrathecally were therefore not due to an action in the periphery. However, intravenous infusions of NE (0.06 and 0.10 µmoles) produced hypothermia and a transient tail skin vasodilation. These results indicated that an action at peripheral sites may have contributed to the effects produced by intrathecal injection of this monoamine. Therefore, several experiments were performed to determine the sites and mechanisms of action of NE injected intrathecally.

Following intrathecal injection of ^{3}H-NE, the majority of radioactivity recovered from the CNS was found in the thoraco-lumbar regions of the spinal cord, whereas very little activity was found in the brain. Thus, the thermoregulatory effects of intrathecal NE are not due to an action of this monoamine at supraspinal sites.

The initial hyperthermic component of the biphasic T_c response to intrathecal injection of the 0.30 µmole dose of NE was temporally correlated with the appearance of high levels of unmetabolized NE in the plasma and was not inhibited by mecamylamine-induced ganglionic blockade. These results indicate that this hyperthermic response was

due to a direct action of NE at peripheral sites following leakage from the spinal subarachnoid space.

Regardless of the dose, unmetabolized NE was present in the plasma during the hypothermia caused by intrathecal injection of NE. Whether these plasma concentrations of NE contribute to the hypothermia was not determined in this study. However, it seems likely that only the relatively large concentrations (e.g., 10 ng/ml) of NE in the plasma following intrathecal injection of the highest dose (0.30 µmoles) could be partially responsible for the fall in T_c caused by this dose of NE.

Clonidine (0.035 µmoles) and NE (0.30 µmoles) injected intrathecally at the level of the lumbar enlargement decreased neural activity recorded from the lumbar sympathetic chain. That this decrease in sympathetic outflow was mediated by an action of NE in the intermediolateral nucleus (IML) of the cord was demonstrated in experiments where rats were prepared with spinal catheters of modified lengths such that the accessibility of intrathecal NE to the IML was altered. NE injections through these modified catheters elicited thermoregulatory effects which differed from those elicited by injections near the lumbar enlargement.

These results suggest that the hypothermia produced by intrathecal injection of NE was mediated primarily by a

direct action of this monoamine at the IML, causing a reduction of sympathetic outflow. However, an action of NE in the periphery following leakage from the subarachnoid site of injection might augment the developing hypothermia and is probably totally responsible for the hyperthermia associated with the 0.30 μmole dose of intrathecal NE.

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TABLE OF CONTENTS

Page

Page

Page

Page

LIST OF ILLUSTRATIONS

Figure — Page

LIST OF TABLES

I. INTRODUCTION

A. The Involvement of the Anterior Hypothalamus-Preoptic Region (AH/PO) in Thermoregulation

Regardless of the ambient temperature, the body temperature of mammals is regulated within a restricted range. This fact necessitates the possession by mammals of a responsive thermoregulatory system capable of initiating appropriate, coordinated changes in physiological effector mechanisms such that body temperature is maintained in the face of an environmental challenge. Three types of physiological effector (thermoeffector) mechanism are available to promote a loss or gain of body heat: 1) Heat can be produced through metabolic processes such as mobilization of free fatty acids (non-shivering thermogenesis) or by shivering; 2) Vasomotor tone can be modified to promote heat retention or to promote the transfer of heat from the core to the skin where it is lost to the environment; and 3) Body heat can be lost by sweating, panting, or wetting the fur with saliva (evaporative heat loss). In addition to these physiological mechanisms, behavior is also involved in thermoregulation. Thus, an animal will actively seek a thermoneutral environment.

Historically, the activities of these thermoeffector mechanisms have been thought to be under the control of the

anterior hypothalamus and adjacent preoptic region (AH/PO) (Hammel, 1968; Hardy et al., 1970; Gale, 1973). The AH/PO receives afferent thermal information from thermodetectors located within the confines of this nuclear complex and other areas of the central nervous system (CNS) (i.e., medulla, spinal cord) and in the abdomen, skeletal muscles, and skin (Cabanac, 1975). The information supplied by these thermal feedback circuits is integrated by the AH/PO, and appropriate changes are initiated in behavior and the activity of various thermoeffector mechanisms.

Several heuristic models have been developed to describe the mammalian thermoregulatory system (Hammel, 1968; Bligh, 1979) which behaves as if it were programmed with some kind of set-point or reference temperature. The set-point temperature is the body temperature for a given mammalian species at which physiological processes (e.g., metabolism) function at optimal levels. That the reference signal (or temperature) has a neuronal basis has not been conclusively determined to date. Regardless of this fact, according to some of the above models information concerning the core temperature, received from deep body thermodetectors, is compared to the set-point temperature and, if a difference (load error) exists, the AH/PO then initiates the appropriate thermoeffector response pattern. Under these conditions, alterations in core temperature

would have to occur before changes in thermoeffector mechanisms were instigated. However, it is well known that mammalian body temperature is maintained despite fluctuations in the ambient temperature. Thus, the thermoregulatory system is more complex than implied above. The AH/PO receives information concerning the ambient temperature from cutaneous thermodetectors. Based on these incoming signals, the AH/PO can predict and subsequently initiate appropriate changes in thermoeffector activities before changes in core temperature can be induced by a non-thermoneutral environment.

In summary, the AH/PO nuclear complex appears to be the integrating controller of mammalian body temperature. Moreover, by monitoring the internal and external thermal environment, and by initiating suitable reflexive changes in peripheral thermoeffectors, core temperature is closely regulated within strict limits.

B. The Role of Hypothalamic Neurotransmitters in Controlling Body Temperature

In 1943, von Euler and colleagues found that injections of epinephrine (Epi) into the cerebral ventricles of rats produced hyperthermia. That Epi, norepinephrine (NE), and 5-hydroxytryptamine (5-HT) might serve as neurotransmitters in the hypothalamus was suggested by the findings

of Vogt (1954) and Amin et al. (1954). These authors showed that the catecholamines and 5-HT were present in extracts of the hypothalamus. In 1961, von Euler made the following suggestion:

> It is currently believed that catecholamines, 5-HT, or both may be mediators of activity of brain-stem structures and involved in the regulation of somatic, autonomic, and cortical activity. It is inviting, therefore, to think that these putative central neurohumours, as well as drugs interfering with their actions of metabolism, might also exert an influence on the setting mechanism of the body thermostat.

Von Euler's suggestion was supported by the data of Feldberg and Myers (1963). These authors discovered that in cats, intracerebroventricular (IVT) injections of Epi and NE caused hypothermia, whereas similar injections of 5-HT elicited hyperthermia. Based on these findings, Feldberg and Myers (1963) proposed that body temperature was controlled by a balance between these opposing thermoregulatory actions due to the relative rates of release of the catecholamines and 5-HT. This hypothesis was made more tenable by the findings that monoamines were located mainly within nerve fibers and terminals of the hypothalamus (Carlsson et al., 1962; Dahlstrom and Fuxe, 1964; Anden et al., 1965). These data were consistent with a neurotransmitter role for the monoamines.

Since the Feldberg-Myers theory, numerous studies have been conducted in a variety of species to determine the

thermoregulatory significance of the hypothalamic monoamines (Clark and Clark, 1980a). Recent neuroanatomical and histochemical studies (Kent and Sladek, 1978; Palkovits et al., 1980; Sawyer and Clifton, 1980; West and Fillenz, 1980) have confirmed previous data concerning the presence of Epi, NE, and 5-HT in various hypothalamic nuclei and have also shown that cholinergic pathways terminate in this region of the brain (Shute and Lewis, 1968; Kobayashi et al., 1978; Lewis and Shute, 1978). The latter finding has prompted studies concerning the involvement of acetylcholine (ACh) in thermoregulation (Clark and Clark, 1980b).

Drugs (e.g., NE, 5-HT) or biological products (e.g., endogenous pyrogen) can produce changes in thermoregulation by two mechanisms. The agent can alter body temperature by resetting the reference temperature of the AH/PO. Perhaps the best example of this is the hyperthermia produced by the central actions of endogenous pyrogens. The set-point of the AH/PO is elevated by the pyrogen which results in a positive load error. In response to the load error, a coordinated modulation of thermogenic and heat dissipation effector mechanisms occur to produce a rapid increase in heat storage. Thus, in the rat during the rising phase of the pyrogen-induced hyperthermia, shivering and peripheral vasoconstriction would be observed along with behavioral activity such as huddling. Body temperature will increase

until the level of thermoregulation is reached commensurate with the temperature dictated by the set-point. The "new" body temperature will be maintained within narrow limits by simple adjustments of vasomotor tone and behavioral thermoregulation. Since the febrile body temperature is regulated by the executive controller, it will be defended as efficaciously and as precisely as is the normal core temperature. If transient thermal stresses produce a displacement of core temperature from the regulated level, following termination of the stress, core temperature will be returned rapidly and accurately to the level existing prior to application of the stress.

Conversely, biological and pharmacological agents can change body temperature by tonic activation and/or inhibition of one or more thermoeffector systems (e.g., shivering or vasomotor tone). The alteration in core temperature observed here is not a result of a change in set-point and exhibits several distinct functional characteristics. Under these circumstances, the core temperature has deviated from the regulated range imposed by the AH/PO set-point. This sets up a negative load error and initiates compensatory activation or inhibition of the non-driven thermoeffector systems. In the rat, a hyperthermia produced in this manner would be accompanied by tail skin vasodilation and behavioral activity such as spreading

saliva on the fur. All these responses constitute an attempt to decrease the load error. The final body temperature reached during the plateau stage would represent an equilibrium between the driven thermoeffector system(s) and whatever compensatory effector activities remain under the control of the AH/PO. A thermoeffector system which is tonically driven would not be responsive to changes in the ambient temperature. Consequently, the magnitude of the change in body temperature would be influenced by the environmental temperature. The plateau level of thermoregulation produced by a driven thermoeffector would be defended poorly because it does not represent a regulated interaction of thermoeffectors mediated by the controller, but rather an equilibrium between the thermoeffectors. Perturbations of the plateau core temperature might augment or inhibit the compensatory effector actions but would not alter the status of the drug-induced dysfunctional thermoeffector system(s). Thus, any perturbation (either internal or external) of the plateau stage would not be vigorously opposed, and body temperature would only slowly drift back to the pre-perturbation level.

It is obvious from the discussion above that several functional characteristics separate those agents which produce changes in body temperature by resetting the reference temperature of the controller from those which alter core

temperature by tonically driving thermoeffector system(s). The following is a comparison of these characteristics:

Drug-induced change in set-point

1. Produces a positive load error which initiates both autonomic and behavioral mechanisms responding in concert to produce a rapid change in body temperature
2. "New" body temperature is maintained by simple adjustments of vasomotor tone and magnitude of change in body temperature is not susceptible to ambient temperature
3. "New" body temperature is regulated and perturbations are vigorously opposed

Drug-induced driven thermoeffector(s)

1. Produces a negative load error which initiates compensatory activation of nondriven thermoeffectors to decrease the error and return body temperature to normal levels
2. "New" body temperature reflects an equilibrium between nondriven and compensatory thermoeffectors and the magnitude of temperature change is altered by the ambient temperature
3. "New" body temperature is unregulated and defended poorly against perturbations

In rats, central injection of several cholinergic agonists (e.g., ACh, carbachol) have been shown to produce a fall in body temperature by lowering the set-point (Satinoff, 1979). The opposite conclusion was reached concerning the effects of centrally administered 5-HT; injections of this indoleamine into the AH/PO caused a rise in body temperature, whereas 5-HT injected into the ventricular system produced hypothermia. Neither thermoregulatory effect was mediated by a change in set-point (Bruinvels, 1970; Crawshaw, 1972). The findings concerning the ability of NE to modify set-point are complex. Low doses of NE (0.45-1.35 µg) injected into the AH/PO have been shown to increase core temperature by raising the set-point (Beckman, 1970; Satinoff and Hackett, 1977). In contrast, larger doses of NE (25 µg) injected similarly caused a fall in body temperature which was mediated by drug-induced driven thermoeffectors (Avery and Penn, 1973; Satinoff and Hackett, 1977). A similar conclusion was reached concerning the hypothermia induced by IVT injections of NE; the thermoregulatory effect of NE given by this route was not caused by a drug-induced fall in the set-point (Satinoff, 1979). In summary, injections of cholinergic agonists and low doses of NE into the rat AH/PO can alter the thermoregulatory set-point, whereas the changes in body temperature induced by AH/PO injections of 5-HT appear to

be mediated by a direct action of this indoleamine on thermoeffector mechanisms.

C. Evidence for the Existence of Extrahypothalamic Sites Involved in Thermoregulation

A large portion of the studies concerning neurotransmitters and thermoregulation have used the IVT or intracisternal (IC) route of injection for drugs. The rationale for the use of these routes of administration was based on the assumption that any observed change in thermoregulation was mediated by an action of the drug at the AH/PO. However, many of the investigations employing the IVT or IC routes used large, nonphysiological volumes of injections. Under these conditions, the chances for hypothalamic localization are minimized and distribution of the drug along the entire ventricular system becomes highly probable. Indeed, several studies have determined the cerebroventricular distribution pattern of dyes or radiolabeled drugs (e.g., ^{3}H-NE) following their IVT or IC injection in volumes comparable to those used in the aforementioned thermoregulatory investigations. These results indicate that these agents so injected distribute along the ventricular system and subsequently reach the spinal subarachnoid space (Glowinski et al., 1965; Schonberg et al., 1967; Banerjee et al., 1970; Calmers and Wurtman, 1971;

Nowaczyk et al., 1978).

In thermoregulatory studies, Crawshaw (1972) has shown that intrahypothalamic (IH) injections of 5-HT (0.05-1.0 µg) in rats caused dose-dependent increases in core temperature, whereas IVT injections of this monoamine (10 µg) produced hypothermia. Crawshaw (1972) concluded that the thermoregulatory effects of IVT 5-HT were not mediated by an action on the AH/PO and therefore, 5-HT injected IVT must have acted at paraventricular structures such as the area postrema of the fourth ventricle. If a drug injected IVT produced its thermoregulatory effects by acting at the AH/PO, then ablation of this area should prevent any changes in body temperature by this drug. However, Cantor and Satinoff (1976) found that in rats with hypothalamic lesions the hypothermia caused by IVT injection of NE was enhanced rather than abolished. Thus, the above discussion challenges the historical view of the AH/PO as the sole center of thermoregulation and suggests that other paraventricular areas of the neuroaxis and their respective monoaminergic pathways and terminals might constitute extrahypothalamic sites importantly involved in the process of regulating core temperature.

Experiments investigating the thermoregulatory capabilities of hypothalamic lesioned rats provide further evidence which suggest that the AH/PO might not be the only

CNS center involved in the control of core temperature. Research has shown that in hypothalamic lesioned rats, a considerable degree of thermoregulatory capability or, at most, only differential impairments of thermoeffector responses exist (Lipton, 1968, 1973; Satinoff and Rutstein, 1970; Satinoff and Shan, 1971; Toth, 1973). These results indicate that CNS structures other than the hypothalamus must be involved in controlling body temperature. Simon (1974) has suggested that the temperature regulation system as a whole may be characterized as being composed of multiple controllers which might, in principle, function independently of each other. Studies in spinalized animals have shown that these preparations retain rudimentary thermoregulatory responses (e.g., shivering) (vide infra). Thus the spinal cord is capable of some independent thermoregulatory actions and appears to be a likely candidate as an extrahypothalamic controller of body temperature.

D. The Involvement of the Spinal Cord in Thermoregulation: Comparison Between the Spinal Cord and the AH/PO

(i) Thermosensitivity

If other nuclei or regions of the CNS play a significant role in regulating body temperature, one would expect these sites to exhibit some of the characteristics of the AH/PO which are associated with a thermoregulatory

center. The AH/PO is thermosensitive; that is, experimentally induced changes in the local temperature of this region activate or inhibit appropriate physiological responses (e.g., vasomotor tone, metabolism) in unanesthetized animals. Neurons have been located in the AH/PO which have a Q_{10} greater than 2 (high Q_{10} thermosensor) and which are most likely the neuronal basis of the observed thermosensitivity of this region (Hensel, 1973). The spinal cord also exhibits thermosensitivity. Simon et al. (1963) were the first to demonstrate that when the lower thoracic-upper lumbar region of the spinal cord of the anesthetized dog was cooled by means of a water perfused peridural thermode, shivering and increased oxygen consumption occurred even at high ambient temperatures. Rautenberg and Simon (1964) showed that, in dogs, shivering induced by low ambient temperatures could be augmented by spinal cord cooling or reduced by warming of the cord. Studies of unanesthetized dogs at constant ambient temperatures demonstrated that the spinal cord and AH/PO were functionally equipotent as sensors of core temperature (Jessen and Mayer, 1971). Subsequent research using a variety of laboratory animals (e.g., rabbits, rats, pigeons) has shown that increasing or decreasing the temperature of the cord instigates appropriate physiological and behavioral changes (Simon, 1974).

One might argue that the thermosensitivity of the cord was a function of thermodetector units in non-spinal tissues (e.g., meningeal blood vessels) which were coincidentally activated by the spinal thermode. Pierau (1971) has identified ventral horn α-motoneurons as being thermosensitive; a decrease in cellular temperature increased their excitability, whereas a rise in the temperature of the α-motoneurons decrease their activity. However, according to Klussmann and Pierau (1972), thermal stimulation of these spinal neurons could only account for changes in shivering and could not account for other physiological changes (e.g., vasoconstriction) observed following thermal stimulation of the cord. Simon et al. (1964) have provided evidence that thermosensitive neurons which mediate a wide spectrum of thermoeffector changes are more or less equally distributed over the entire length of the cord. When the temperature of the spinal cord of small animals such as the rabbit or rat is altered, thermoeffector responses (e.g., shivering, changes in vasomotor tone) occur within seconds of the thermal stimulus (Kosaka et al., 1967; Lin and Chai, 1974). This rapid response time indicates that the stimulated thermosensitive structures are located in the superficial aspects of the spinal cord (Simon, 1974). Further support for the spinal localization of these thermodetectors is supplied by the data of Meurer et al. (1967). In

dogs with lumbrosacral dorsal rhizotomies, selective cooling of the deafferentiated section of the spinal cord was still effective in evoking physiological changes. In support of these data, Klussmann (1969) showed that thermal stimulation of the spinal cord did not increase the activity of afferent dorsal root fibers. Moreover, several lines of evidence imply that warm-sensitive neurons are present in the cervico-thoracic region of the guinea pig cord and, that these neurons probably project to the posterior hypothalamus via the spinothalamic tract (Wunnenberg and Bruck, 1970). Based on these data, it seems reasonable to suggest that spinal thermosensitivity is a function of temperature-sensitive neurons or neural circuits similar to those found in the AH/PO (i.e., high Q_{10} thermodetectors). Thus the spinal cord shares the hypothalamus the characteristic of thermosensitivity.

Several investigators have suggested that the effector changes which occur in response to thermal stimulation of the cord depend upon transmission of afferent information via ascending neural projections to the hypothalamus (Kosaka et al., 1969; Gale, 1973). This may not be entirely true. Chai and Lin (1975) have shown that in unanesthetized rabbits, the vasomotor, respiratory, cardiac, and muscular responses to thermal displacement of the medulla oblongata and spinal cord were not altered

following isolation of the AH/PO and other rostral structures by midbrain transsection. These results indicate that the medulla and spinal cord are independent of the AH/PO with respect to thermosensitivity and thermoregulation. Similar results were observed following thermal stimulation of the medulla and cord in rats with AH/PO lesions (Lin and Chai, 1974). However, in these rats, when the medulla was separated from the spinal cord by transsection at C_7, the respiratory and cardiac responses, as well as a portion of the tail vasomotor response to thermal displacement of the cord, were abolished. These findings indicated that a limited number of thermoregulatory responses (i.e., shivering, some vasomotor effects) can be initiated by the cord independent of other CNS areas. The comparatively reduced efficacy of the spinal cord has prompted Bligh (1973) and Simon (1974) to suggest that this area of the CNS represents an extrahypothalamic site of thermoregulation operating at low gain (i.e., coarse control).

(ii) Integrative capacity

The importance of the AH/PO area to thermoregulation is exemplified by the fact that this brain region is thermosensitive and that it receives and integrates afferent information concerning the internal and external

thermal environment and then issues the proper efferent commands (Hardy, 1972). The spinal cord is also thermosensitive and, as the following discussion indicates, the cord possesses the ability to integrate afferent signals. Recent views which portray the spinal cord as an important integrating center for various physiological processes (i.e., thermoregulation) have replaced the concept that the cord acts simply as a conduit for information going to and coming from supraspinal sites. Research has shown that A_δ and C fibers transmit afferent information from cutaneous warm- and cold-thermodetectors to the spinal cord (Perl, 1968; Hensel, 1969; Iggo, 1969). These primary afferent neurons synapse with second order neurons in the marginal layer (lamina I) and substantia gelatinose (laminae II and III) of the dorsal horn grey matter (Angel, 1977). The second order neurons project to ascending spinal pathways, such as the spinothalamic and spinoreticular tracts, which convey thermal and nociceptive information to supraspinal sites (Wunnenberg and Bruck, 1970; Simon and Iriki, 1971; Angel, 1977).

Research indicates that afferent nociceptive and non-nociceptive thermal information is processed in the dorsal horn prior to being forwarded to various brain areas (Christensen and Perl, 1970; Hellon and Misra, 1973; Price and Browe, 1975; Necker and Hellon, 1978). For example,

Hellon and Misra (1973) showed that the responses of thermodetector neurons in the rat scrotum to thermal stimulation differed from the responses of dorsal horn neurons to the same stimulation applied to the scrotum. Based on these data and other evidence, the authors concluded that considerable integration of incoming thermal data must be occurring in the dorsal horn. The modulation of afferent thermal information which takes place in the dorsal horn could involve descending excitatory and inhibitory neuronal input from several brainstem areas (Wall, 1967). Bulbospinal monoaminergic and cholinergic terminals have been found in the dorsal horn, and several investigators have suggested that these descending pathways might play a role in the integration of sensory input at the spinal level (vide infra).

Integration is also an important aspect of efferent spinal cord functions. Preganglionic sympathetic neurons of the intermediolateral nucleus (IML) of the spinal cord receive both inhibitory and excitatory neuronal inputs from supraspinal areas including direct input from several hypothalamic nuclei (Coote and Macleod, 1974; Gebber and McCall, 1976; Saper et al., 1976; Sofroniew and Schrell, 1980; Swanson and Kuypers, 1980). Thus, the efferent activity of the preganglionic neurons and, ultimately, the level of peripheral sympathetic tone are a function of integration

occurring at the level of the IML (Coote and Macleod, 1974; Hilton and Spyer, 1980). Regarding the production of shivering, Klussmann and Pierau (1972) have suggested that the α-motoneuron represents the final integrating system for signals from thermodetector units located in other areas of the CNS and periphery. Thus, the intensity of shivering is dependent upon integration at the level of the α-motoneurons.

The preceding discussion demonstrates that the processing of various types of afferent and efferent information relevant to thermoregulation occurs at the level of the spinal cord. Therefore, not only does the cord share with the AH/PO the attribute of thermosensitivity, but also both areas of the CNS exhibit the ability to integrate thermoregulatory information.

(iii) Monoaminergic and cholinergic innervation

The spinal cord and AH/PO exhibit another common trait; both are heavily innervated by monoaminergic and cholinergic fibers. The innervation of the hypothalamus has been discussed above. Various areas of the spinal grey matter receive descending monoaminergic and cholinergic pathways from several brainstem nuclei. In the following section, the location of brainstem cell bodies of origin, the spinal tracts, and the terminal fields in the grey

matter will be identified for each bulbospinal system.

The bulbospinal noradrenergic system. The rat spinal cord receives descending input from several pontine and medullary nuclei which contain the cell bodies of noradrenergic neurons. The A_1 (designation according to Dahlstrom and Fuxe, 1964) grouping of noradrenergic cell bodies is located on the ventral aspect of the medulla, and coincides with the "vasopressor area" of this brain region. The A_2 cell bodies are contained within the region of the nucleus tractus solitarius. The A_5 nucleus appears to be a rostral extension of the A_1 cells and is situated lateral to the superior olivary nucleus and medial to the roots of the seventh and fifth cranial nerves. Axons from the A_1, A_2, and A_5 nuclei descend through the spinal cord along the dorsolateral funiculus (DLF) (Carlsson et al., 1964; Dahlstrom and Fuxe, 1965; Ungerstedt, 1971; Lindvall and Bjorklund, 1974; Satoh et al., 1977). The spinal cord of the rat also receives noradrenergic input from the locus coeruleus (A_6) (Nygren and Olson, 1977; Satoh et al., 1977; Guyenet, 1980). The coeruleospinal pathway is located in the anterior funiculus and the ventral part of the lateral funiculus (Nygren and Olson, 1977).

Histochemical and immunocytochemical studies have shown that the pontine and medullary noradrenergic

projections terminate primarily in the grey matter of the cord. In contrast, very few terminals have been observed in the white matter of the cord (Zivin et al., 1975). Noradrenergic terminals have been identified in dorsal horn laminae of the grey matter (Carlsson et al., 1964; Zivin et al., 1975). These laminae receive the majority of their noradrenergic input from the locus coeruleus, with some terminals being supplied by the A_1 and A_2 nuclei (Carlsson et al., 1964; Zivin et al., 1975). Fluorescent histochemical and immunocytochemical studies have shown that the IML is densely innervated by noradrenergic fibers (Carlsson et al., 1964; Fuxe, 1965; Zivin et al., 1975; Commissiong et al., 1978; Glazer and Ross, 1980) which stem from medullary A_1, A_2, and A_5 nuclei (Dahlstrom and Fuxe, 1965; Nygren and Olson, 1977; Loewi et al., 1979). These bulbospinal noradrenergic terminals appear to make intimate synaptic contact with preganglionic neurons in the IML (Dahlstrom and Fuxe, 1965). The ventral horn of the grey matter receives dense noradrenergic terminations from cell bodies located in the ventral aspects of the locus coeruleus (Nygren and Olson, 1977; Commissiong et al., 1978). Histofluorescence studies have identified rosettes of bulbospinal noradrenergic varicosities surrounding α-motoneurons (Carlsson et al., 1964; Zivin et al., 1975; Commissiong et al., 1980). Thus, most areas of the spinal grey matter receive noradrenergic

input from several brainstem nuclei.

The bulbospinal serotonergic system. The cell bodies of the CNS serotonergic pathways are located primarily in the mesencephalic and medullary raphe nuclei (Dahlstrom and Fuxe, 1964). The mesencephalic raphe nuclei appear to be the origin of an ascending serotonergic system (Anden et al., 1966), whereas axons from the medullary nuclei project to the spinal cord (Dahlstrom and Fuxe, 1965). These latter raphe nuclei are the nucleus raphe magnus (B_3), nucleus raphe obscurus (B_2), and the nucleus raphe pallidus (B_1). The axons of the raphe magnus descend along the DLF, whereas the raphe obscurus and pallidus axons course through the cord via the ventrolateral fasciculi and ventral funiculi (Basbaum et al., 1978; Leichnetz et al., 1978; Martin et al., 1978; Basbaum and Fields, 1979).

The spinal cord grey matter receives abundant serotonergic nerve terminals from the raphe nuclei. Particularly high concentrations of 5-HT varicosities are found in the dorsal horn, the IML, the motoneuron area of the ventral horn, and in the central area bordering the ependymal canal (Dahlstrom and Fuxe, 1964; Zivin et al., 1975; Oliveras et al., 1977). Oliveras et al. (1977) found that lesions of the raphe magnus produced a relatively selective reduction in the levels of 5-HT in the substantia gelatinosa of the

dorsal horn. These data suggest that this area receives the majority of its serotonergic input from the raphe magnus.

The bulbospinal cholinergic system. The bulbospinal cholinergic system has not been characterized as completely as the monoaminergic system. Histochemical studies have shown that in several species ACh, cholineacetyl-transferase, and acetylcholinesterase are present in the dorsal and ventral horns and in the IML of the grey matter (MacIntosh, 1941; Navaratnam and Lewis, 1970; Kasa, 1975; Lewis and Shute, 1978). However, the cholinergic cell bodies of origin and corresponding spinal pathways have not been adequately identified. Gwyn and Wolstencroft (1966) have suggested that cholinergic cell bodies in the pontine reticular formation might project axons to the various spinal areas, but this suggestion has not been confirmed.

The previous sections indicate that bulbospinal monoaminergic and cholinergic fibers terminate in the dorsal and ventral horns and the IML of the grey matter. As indicated earlier, these areas of the spinal cord are also involved in the processing of information pertinent to the control of body temperature. It is possible that noradrenergic, serotonergic, and cholinergic synapses in the dorsal and ventral horns and the IML play an important role in the

integration of information related to thermoregulation.

E. Statement of the Problem

The material presented in the Introduction indicates that CNS areas other than the AH/PO play an important role in controlling body temperature. Simon (1974) has proposed that the spinal cord represents an extrahypothalamic site of thermoregulatory function. Indeed, the cord exhibits many of the characteristics associated with a thermoregulatory center. The spinal cord, like the AH/PO, possesses a population of thermodetector units. Thermal stimulation of these units produces changes in the status of various peripheral thermoeffectors. Some of these changes are organized at the spinal level (e.g., shivering). A region of the CNS which is actively involved in determining body temperature must be capable of integrating arriving neuronal signals. Such an ability has been demonstrated for the cord. Information pertinent to thermoregulation is processed in the dorsal and ventral horns and the IML of the grey matter before it is forwarded to the brain or periphery. Therefore, the cord shares with the AH/PO the attributes of thermosensitivity and integrative capacity, and is importantly involved in the regulation of body temperature.

The AH/PO region is heavily innervated by

monoaminergic and cholinergic fibers, and the role of these hypothalamic neural systems in thermoregulation has been investigated extensively. Monoaminergic and cholinergic pathways also innervate several areas of the spinal cord. A role for these spinal pathways in thermoregulation is suggested by the fact that the regional distribution of their respective nerve terminals coincides with specific spinal grey matter areas which are involved in the control of body temperature. In spite of the possible function of these spinal pathways in thermoregulation, no investigations of their putative roles have been carried out.

The purpose of the present study was to examine the possible involvement of spinal cord monoaminergic and cholinergic systems in rat thermoregulation. Drugs used in this study were injected into the spinal subarachnoid space via chronic indwelling catheters extending to the level of the lumbar enlargement.

II. OVERVIEW OF EXPERIMENTAL DESIGN

The present series of experiments has been divided into two phases. In Phase I, the effects on thermoregulation were determined following injection of the cholinergic agonist carbamylcholine (CCh), and the adrenergic agonists, NE and clonidine into the rat spinal subarachnoid space via catheters extending to the lumbar enlargement. To demonstrate whether the agents injected intrathecally might produce their thermoregulatory effects by acting at peripheral sites following leakage from the subarachnoid site of injection, several doses of NE, 5-HT, and CCh were administered intravenously. Intravenous injections of 5-HT and CCh did not alter thermoregulation, whereas intravenous (i.v.) and intraperitoneal (i.p.) administration of NE produced changes in core temperature and tail skin temperature similar to those changes induced by intrathecal NE. These data suggested that an action of NE at peripheral sites may have contributed to the effects caused by intrathecal injection of this monoamine. Therefore, Phase II experiments were designed to determine the sites and mechanisms of action for NE injected intrathecally.

In Phase II, four independent experiments were carried out. In the first, to ascertain whether appreciable amounts of intrathecally injected NE reach the supraspinal

centers and/or the peripheral sites, radiolabeled NE was injected intrathecally, and the distribution of label in the brain and blood was determined. The highest dose of NE injected intrathecally (0.3 μmole) produced an initial hyperthermia followed by hypothermia. Therefore, in the second experiment, the possibility that the hyperthermic effects of the high dose of intrathecal NE was due to direct stimulation of peripheral receptors mediating non-shivering thermogenesis (NST) was tested by injecting NE intrathecally in rats pretreated with the ganglionic blocker, mecamylamine. The thermoregulatory changes induced by intrathecal injection of NE and clonidine suggested that these drugs might act by inhibiting sympathetic outflow from the thoraco-lumbar IML. To test this hypothesis, the effects of intrathecal NE and clonidine on neural activity recorded from the lumbar sympathetic chain were determined. In the final facet of Phase II, rats were prepared with spinal catheters which extended to either the upper cervical area or the lower sacral region of the spinal cord. If the IML mediates the hypothermic effects of intrathecal NE, injection of NE near this nucleus (via the lumbar catheters) should have a greater effect than injections at distant loci (via cervical and sacral catheters).

III. METHODS

A. Animals

Male Holtzman rats weighing 300-350 gm at the time of surgery were used. The rats were housed individually in a room maintained at 23° ± 1°C having a 12 hour light-dark cycle. Food and water were available ad libitum.

B. Drugs

Drugs used were l-norepinephrine bitartrate (NE), 5-hydroxytryptamine hydrochloride (5-HT), carbamylcholine chloride (CCh), and mecamylamine hydrochloride (Sigma Chemical Company, St. Louis, MO); d-norepinephrine bitartrate (d-NE) (Adams Chemical Company); clonidine hydrochloride (Boehringer-Ingelheim Ltd., Ridgefield, CT); and levo-[7,8-^{3}H(N)]-norepinephrine (^{3}H-NE) (New England Nuclear, Boston, MA, 30.6 Ci/μmole). Drugs injected intrathecally were dissolved in an artificial cerebrospinal fluid (ACSF) described by Yeung and Rudy (1980). Drugs injected intraperitoneally or intravenously were dissolved in 0.9% sterile saline. Drug solutions were prepared daily.

C. General Surgical Procedures

(i) Catheterization of the spinal subarachnoid space

Rats which were to receive intrathecal injections were anesthetized with pentobarbital and prepared with chronic indwelling spinal catheters according to the method of LoPachin et al. (1981). In the majority of experiments, the catheter was advanced 8.5 cm beyond the atlanto-occipital membrane into the subarachnoid space. This places the tip of the catheter just rostrad to the lumbar enlargement. However, in one study, rats were prepared with catheters which extended either 4 cm or 12 cm beyond the atlanto-occipital membrane. Rats were allowed to recover from surgery for at least one week before they were used in an experiment. At the conclusion of all experiments, the rats were sacrificed with pentobarbital, and the position of the spinal catheter within the subarachnoid space was determined.

(ii) Intravenous catheterization

In those experiments in which intravenous injections were required, the injections were made through chronic intravenous catheters fashioned from polyethylene tubing (PE10). The right external jugular vein was exposed by a small incision in the neck. The catheter was inserted through a vein to the junction of the vena cava with the

right atrium. The catheter was tied into the vein, and the free end of the tubing was brought subcutaneously to exit through a stab wound at the nape of the neck. The rats were allowed to recover from surgery for a week. Daily injections of heparinized saline maintained the patency of the catheter.

(iii) Catheterization of the peritoneal cavity

In some experiments, rats were prepared with intraperitoneal catheters. The catheters facilitated intraperitoneal administration of drugs to restrained rats during experiments where colonic temperature (T_c) and tail skin temperature (T_{sk}) were recorded. The catheter was fashioned from a length of polyethylene tubing (PE10). One end of the tubing was formed into a permanent planar coil and inserted into the peritoneal cavity through a small incision in the lower right abdominal quadrant of an anesthetized rat. The free end of the catheter was brought subcutaneously to exit through a stab wound at the nape of the neck. The catheter was rinsed with saline and occluded with a small stylet. Rats were allowed to recover for one week. The patency of the catheter was maintained by daily injections of saline.

D. Injection Techniques

Intrathecal injections of control and drug solutions were carried out using a 10 µl Hamilton syringe with a 30 ga permanently affixed needle. The syringe was attached directly to the exterior portion of the spinal catheter and was driven by a hand-held micrometric syringe buret. Drug and control solutions were injected into the spinal subarachnoid space in a volume of 4 µl. Intrathecal injections required approximately two minutes to complete.

As a control for the intrathecal injections of norepinephrine bitartrate, a solution of sodium bitartrate (Fisher Scientific Company, Fair Town, NJ) was prepared in sterile ACSF. The pH, osmolarity, and bitartrate concentration of this solution were adjusted to match those of the highest dose of NE used in these experiments (0.30 µmoles NE = 605 mosmoles, pH = 3.7). The control solutions for the chloride ion, pH, and osmolarity of the 5-HT, CCh, and clonidine solutions were prepared by adding sufficient sodium chloride to a volume of ACSF to make the solution isotonic with respect to the highest dose of drug used (0.90 µmoles 5-HT = 609 mosmoles, 0.06 µmoles CCh = 274 mosmoles, 0.07 µmoles clonidine = 290 mosmoles). The final solution was brought to the appropriate pH with 0.05 N HCl (5-HT = pH 2.77, CCh = pH 6.2, clonidine = pH 6.5).

The prepared solutions to be injected were sterilized

by forcing them through a millipore filter unit (Millex^{R}--GS, 0.22 μm filter unit). The solutions were received into sterilized test tubes and used within 30 minutes. The Hamilton glass microliter syringes used for intrathecal and intravenous injections were stored in 95% alcohol.

Intravenous injections of control and drug solutions were carried out using a gear driven Hamilton microliter syringe connected to the intravenous catheter by a length of PE10 tubing. Drugs were dissolved in sterile saline, filtered (vide supra), and injected in a volume of 100 μl over periods of 5, 10, or 15 minutes. Intravenous injections of sterile saline (100 μl) served as a control.

Several doses of NE were injected intraperitoneally in restrained rats. The dose of NE was dissolved in 0.5 ml of sterile saline and injected into the intraperitoneal catheter. A subsequent injection of sterile saline (0.2 ml) washed any residual drug from the catheter into the peritoneum. Intraperitoneal injection of sterile saline (0.5 ml) served as control.

E. Recording of Physiological Responses

T_c and T_{sk} were continuously monitored throughout each experimental session. During some sessions electromyographic (e.m.g.) activity was recorded as an index of shivering. To measure T_c, a YSI 401 thermistor probe

(Yellow Springs Instrument Company) was inserted 6 cm into the colon. T_{sk} was assessed with a YSI 427 thermistor disk applied to the ventral surface of the tail approximately 2 cm from the anus. The tail and colonic thermistors were held in place by taping the leads to the rat's tail. T_c and T_{sk} were continuously recorded using a multichannel potentiometric recorder.

In some experiments, the e.m.g. activity of the lateral thigh muscles of one hind leg was recorded according to the method of Ackerman and Rudy (1980). At the beginning of each experimental session in which e.m.g. activity was recorded, the rat was warmed with an infrared light to suppress shivering. The residual background activity was subtracted from the e.m.g. values recorded during the session. Intrathecal injections of NE occasionally produced myoclonic contractions which contaminated the e.m.g. records (vide infra). A combination of electronic and manual adjustment of the data were used to subtract out artifacts arising from these myoclonic jerks.

E.m.g. data were acquired as both raw e.m.g. tracings and as integrated e.m.g. activity. The raw tracings were used to monitor the qualitative aspects of the e.m.g. activity. Throughout the text "shivering-like e.m.g. activity" refers to drug-induced changes in e.m.g. tracings which were qualitatively similar to e.m.g. tracings

associated with cold-induced shivering. Integrated e.m.g. was averaged over 2 minute periods and provided an estimate of the quantity of shivering-like activity per unit time. Integrated e.m.g. was reported in arbitrary units of mm pen rise per minute.

All experiments were performed at an ambient temperature of 23° ± 1°C. On the day of an experiment, rats were loosely restrained in wire mesh cages to which they had been previously acclimated. Thermistors and recording electrodes were attached, and baseline data were recorded for at least an hour. Drug or control solutions were injected, and the physiological parameters discussed above were recorded for the duration of the treatment-induced effects or until it was evident that the treatment did not elicit changes in thermoregulation.

F. Quantification of Thermoregulatory Responses

The ability of drugs injected intrathecally to produce changes in rat thermoregulation were expressed in terms of the following descriptive parameters. ΔT_c (°C): the maximum change in T_c produced by a drug or control treatment (ΔT_c^+ = maximum increase in T_c; ΔT_c^- = maximum decrease in T_c). The change was measured relative to the rat's baseline T_c. TI (°C): the thermal index (TI) was derived by planimetric measurement of the area between the curve

representing the treatment-induced change in T_c or T_{sk} and the extrapolated baseline. RTC (°C/hr): the rate of temperature change (RTC) represents the average rate at which colonic temperature changed during the first 50% of the total drug effect on T_c (RTC^- = rate of temperature decrease; RTC^+ = rate of temperature increase). This is the period during which the rate of T_c change is maximum. ΔT_{sk} (°C): the maximum change in T_{sk} produced by drug or control treatment (ΔT_{sk}^+ = maximum increase in T_{sk}; ΔT_{sk}^- = maximum decrease in T_{sk}). The change was measured relative to the rat's baseline T_{sk}.

G. Determination of Dose-Response Characteristics

Dose-response curves were constructed for the parameters (vide supra) affected by intrathecal injections of NE, clonidine, 5-HT, and CCh. The doses of the drugs used are listed in Table 1.

In Phase I experiments, rats were prepared with spinal catheters and divided into four groups. Each group received one of the drugs listed in Table 1. The rats of a particular drug group were started on a crossover design in which individual animals received intrathecal injections of a control solution and of each of the different doses of the designated drug. The group of rats which received l-norepinephrine (NE) also received a single injection of

Table 1

Drugs and doses injected into the spinal subarachnoid space

	µmoles	µg salt	µg base
l-norepinephrine bitartrate	0.01	3.19	1.69
	0.03	9.58	5.08
	0.06	19.16	10.01
	0.10	31.93	16.92
	0.30	95.79	50.77
5-hydroxytryptamine hydrochloride	0.03	6.38	5.29
	0.10	21.27	17.65
	0.30	63.81	52.96
	0.60	127.62	105.92
	0.90	191.43	158.88
Carbamylcholine chloride	0.001	0.18	0.15
	0.003	0.55	0.44
	0.006	1.10	0.89
	0.01	1.83	1.48
	0.03	5.47	4.42
	0.06	10.96	8.85
Clonidine hydrochloride	0.018	4.00	3.40
	0.035	8.00	6.80
	0.070	16.00	13.60

d-norepinephrine (0.30 µmoles). The control solution and doses of drug were administered according to a randomized schedule. Intrathecal injections in a given rat were separated by at least two days. The data for each dose-response curve were analyzed by a two-way (treatment x rats) analysis of variance (Winer, 1962), and the means for each dose were compared with the 0.05 level significance using Duncan's multiple range test (Freund et al., 1960).

H. Distribution of ^{3}H-NE Following Intrathecal Injection

To ascertain whether appreciable amounts of intrathecally injected NE reach the supraspinal centers and/or the systemic circulation, at several times following intrathecal injection of ^{3}H-NE at 8.5 cm beyond the atlanto-occipital membrane, we determined the distribution of radiolabel along the neuroaxis and the amount of unmetabolized ^{3}H-NE present in the systemic circulation. Rats were prepared with spinal catheters as described previously. After recovery from surgery, the thermoregulatory response to intrathecal injection of 0.30 µmoles NE was determined for each rat. Two days later, rats were divided into three groups. Each rat received an intrathecal injection of 4 µl of a solution containing 50 µCi ^{3}H-NE and 0.30 µmoles unlabeled NE (51 µg base). For each rat of a particular group, a 5 ml sample of blood was withdrawn by percutaneous

intracardiac puncture at a time which corresponded to one of the following three segments of the previously determined intrathecal NE-induced T_c response: 1) When the rate of T_c increase was maximal during the hyperthermia (sacrifice time = 3 minutes for all rats); 2) When the rate of T_c decrease was maximal during the subsequent hypothermia (sacrifice time = 15-25 minutes); 3) When, prior to returning to normal, T_c had stabilized at a level below normal (sacrifice time = 90-120 minutes). Blood samples were collected in heparinized tubes in the presence of sodium metabisulfite (0.5 mg/ml) and immediately centrifuged in the cold. The resulting plasma supernatant was retained for subsequent column chromatography (vide infra). Once the blood sample was taken, the rat was promptly sacrificed by immersion in liquid nitrogen. This procedure, in comparison to other methods of sacrifice, insured that diffusion of the radiolabel along the neuroaxis was halted precisely at the time of sacrifice.

The brain and spinal cord of each rat were quickly removed and dissected. The brain was divided along the rostro-caudal axis into 5 mm thick slabs beginning at a point just rostrad to the first spinal nerve. Beginning at the same point, but moving in a caudad direction, the spinal cord was cut into 1 cm sections. The brain and spinal cord sections were weighed, and the tritium label

was recovered by oxidizing each sample using a Packard Oxidizer (Model 306, 97% recovery). The radioactivity recovered per tissue sample was counted using a Packard Tri-Carb liquid scintillation spectrometer (Model 460-CD), and the ng NE-base per mg wet tissue weight was calculated.

Unmetabolized ^{3}H-NE in the plasma samples was separated from total ^{3}H-NE metabolites by column chromatography according to a modification of the method of Verbeuren et al. (1977). In our system, no attempt was made to quantitate the metabolites individually. Consequently, the Dowex-1 column, which was used by Verbeuren et al. (1977) to separate two of the metabolites of NE (MOPEG, VMA), was not employed in the present investigation. The results from the column chromatography study were used to calculate the ng of unmetabolized NE and metabolized NE per ml of plasma.

In a separate experiment, two rats were injected with 4 µl of a solution containing 50 µCi ^{3}H-NE only (i.e., no unlabeled NE was included in the injectate). At 3 minutes postinjection, both rats were sacrificed, and 5 ml blood samples were taken.

I. Recording of Neural Activity in the Lumbar Sympathetic Chain

Six rats were prepared with chronic indwelling spinal

catheters extending to the level of the lumbar enlargement. The changes in T_c and T_{sk} induced by intrathecal injections of NE (0.3 µmoles) and clonidine (0.035 µmoles) were assessed in four rats and two rats, respectively.

A minimum of two days later, a rat was selected from either the NE group or the clonidine group and anesthetized with urethane (1 gm/Kg, i.p.). The right jugular vein was cannulated for injection of drugs. A lumbar sympathetic chain was exposed by midline abdominal incision, and by retraction of the dorsal aorta and posterior vena cava. The chain was freed, cleared of connective tissue, and was cut. The proximal end of the nerve was mounted on bipolar silver hook electrodes for recording, and the surgical area was bathed in warm mineral oil. Spontaneous sympathetic neural activity was amplified, displayed on an oscilloscope, and photographed on 35 mm film. Neural activity was led off to a rate meter whose output was recorded on a polygraph as an analog voltage which varied with the discharge frequency.

When neural firing rate had stabilized at a baseline level, a control intrathecal injection for either NE or clonidine was made. One hour after administration of control solution, either NE (0.30 µmoles) or clonidine (0.035 µmoles) was injected intrathecally. Neural firing rate was recorded until activity had returned to the preinjection

level. At this point, NE (51 µg or 102 µg) was infused intravenously over a 10 minute period and firing rate was recorded until neural activity had returned to the preinfusion level. At the conclusion of each experiment, an autopsy was performed to determine the location of the spinal catheter. Treatment induced changes in sympathetic neural activity were expressed as a percentage of baseline firing rate.

IV. RESULTS

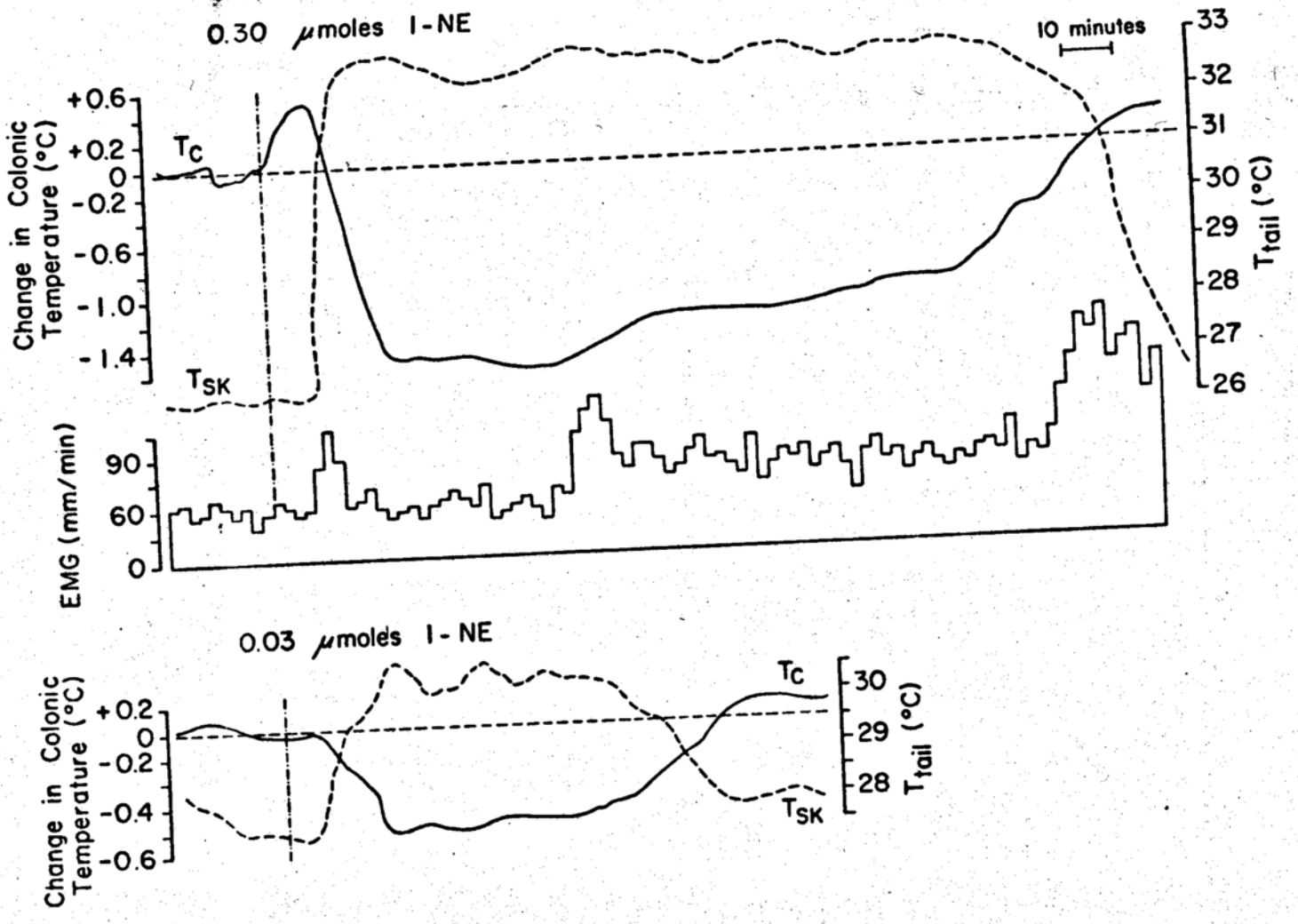
A. Phase I--the Effects of Intrathecal Injections of NE, Clonidine, 5-HT, and CCh on Thermoregulation

(i) General characteristics of the effects of intrathecal adrenergic agonists on thermoregulation

The lowest dose of NE tested (0.01 μmoles) produced no consistent thermoregulatory effects. The major effect of the other doses (0.03-0.30 μmoles) was the production of hypothermia associated with an increase in T_{sk} (Fig. 1). With the 0.03-0.10 μmole doses, T_c began to fall approximately 5-7 minutes after intrathecal injection. At the highest dose (0.30 μmoles), the hypothermia was preceded by a small, transient rise in T_c (0.41 $\pm$ 0.13°C). Thus, at this dose, the start of the hypothermic response was delayed for the duration of the initial hyperthermia (about 20-25 minutes). In 14 sessions (58%), T_{sk} began to rise and T_c began to fall at the same time. During these sessions, the return of T_c and T_{sk} toward normal occurred concomitantly. However, in 10 sessions (42%), the hypothermia was well developed before the change in T_{sk} began. In these experiments, a similar lack of correlation between T_c and T_{sk} was observed during the return of T_c to baseline, i.e., T_c returned to normal in advance of T_{sk}.

In a separate group of rats, the effects of

Figure 1. Example obtained from one rat of the effects on colonic temperature (T_c), tail skin temperature (T_{sk}), and electromyographic (e.m.g.) activity produced by intrathecal injection of the 0.30 µmole dose of NE. Also shown are the 0.03 µmole dose of NE given intrathecally to the same rat. Vertical dashed lines correspond to time of NE injection.


0.30 μmoles I-NE
10 minutes
Change in Colonic Temperature (°C)
+0.6
+0.2
0
-0.2
-0.6
-1.0
-1.4
Tc
TSK
33
32
31
30
29
28
27
26
Ttail (°C)
EMG (mm/min)
90
60
0
0.03 μmoles I-NE
Change in Colonic Temperature (°C)
+0.2
0
-0.2
-0.4
-0.6
Tc
TSK
30
29
28
Ttail (°C)

intrathecal injections of two doses of NE on e.m.g. activity were examined. During these experiments, T_c and T_{sk} were also recorded. Intrathecal administration of control solutions did not alter e.m.g. activity. In four rats given intrathecal injections of 0.10 and 0.30 µmoles of NE, changes in e.m.g. activity did not occur during the hypothermic response until T_c began to return toward normal. The return of T_c to baseline was associated with an increase in shivering-like e.m.g. activity. However, in two other rats given the 0.30 µmole dose of NE, shivering-like activity increased during the fall in T_c and remained above the preinjection level until T_c returned to baseline. It is noteworthy that the initial hyperthermic response produced by the 0.30 µmole dose was not associated with an increase in shivering-like e.m.g. activity, and that a depression of e.m.g. activity did not occur during the fall in T_c produced by either of the doses tested (Fig. 1).

Intrathecal injections of NE control solutions did not alter rat thermoregulation. Moreover, injections of the d-isomer of NE (0.30 µmoles) did not produce significant changes in T_c or T_{sk} in four rats. However, in two rats, intrathecal d-NE produced a small, short-lasting fall in T_c associated with a rise in T_{sk}.

Intrathecal injections of NE elicited several nonthermoregulatory effects. Myoclonic muscle contractions were

observed in some rats at all doses tested. The myoclonic contractions affected the hind limbs and resembled stepping movements. They occurred about 5-8 minutes after NE injection and lasted less than 5 minutes. These muscle contractions were more prevalent at the 0.30 µmole dose of NE. However, they probably did not contribute to the hyperthermia associated with this dose since myoclonus was also seen at doses which did not cause hyperthermia. Athetoid tail movements were also observed after intrathecal injections of NE at all dose levels. Their onset was similar to that of the myoclonic contractions, but they lasted longer (about 15-30 minutes).

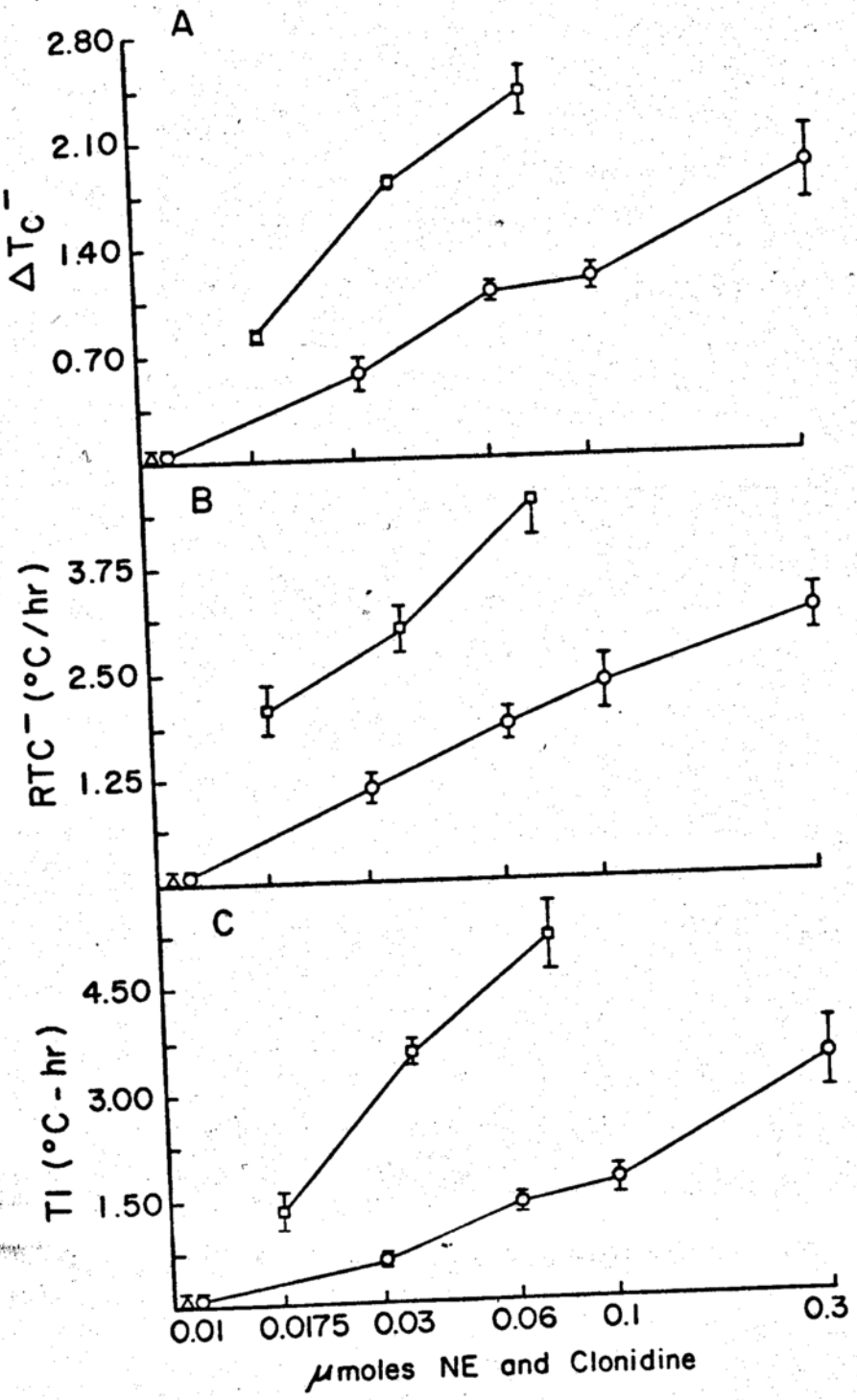
Injections of clonidine (see Table 1 for doses) into the spinal subarachnoid space produced an effect on thermoregulation similar to that produced by intrathecal administration of the 0.03-0.10 µmole doses of NE, i.e., a monophasic hypothermia associates with a prolonged increase in T_{sk}. Intrathecal injections of clonidine control solutions did not alter T_c or T_{sk}. Moreover, nonthermoregulatory changes in behavior or motor function were not observed following intrathecal injection of clonidine.

(ii) Dose-dependency of changes in thermoregulatory responses produced by intrathecal adrenergic agonists

The previous section indicates that a fall in T_c in conjunction with a rise in T_{sk} were the major effects of intrathecal NE and clonidine on rat thermoregulation. For each agonist, statistical analysis indicated that ΔT_c^- (Fig. 2a), the TI of the hypothermia (Fig. 2b) and RTC^- (Fig. 2c) were all dose-dependent. Although both NE and clonidine produced a trend toward a larger ΔT_{sk}^+ with increasing dose, this relationship for either agonist was not statistically significant (data not shown). The ΔT_{sk}^+ for both agonists ranged from 5° to 7°C. However, the duration, and thus the TI, of NE- and clonidine-induced tail skin vasodilation was found to be dose-dependent (data not shown).

(iii) General characteristics of the effects of intrathecal 5-HT on thermoregulation

Intrathecal injection of 5-HT caused an immediate, rapidly developing increase in T_c (Fig. 3; see Table 1 for doses). During the rising phase of the hyperthermia, T_{sk} either decreased slightly or remained unchanged. With the smaller doses of 5-HT (0.03-0.10 μmoles), T_c rose rapidly to a maximum and then immediately began to return


A
2.80
2.10
1.40
0.70
ΔTc⁻
B
3.75
2.50
1.25
RTC⁻ (°C/hr)
C
4.50
3.00
1.50
TI (°C-hr)
0.01
0.0175
0.03
0.06
0.1
0.3
μmoles NE and Clonidine

0.60 μmoles 5-HT
10 minutes
T_c
T_{SK}
T_{tail} (°C)
32
30
28
26
Change in Colonic Temperature (°C)
+0.6
+0.4
+0.2
0
-0.2
EMG (mm/min)
210
140
70
0
0.30 μmoles 5-HT
T_c
T_{SK}
T_{tail} (°C)
27
26
25
Change in Colonic Temperature (°C)
+0.6
+0.4
+0.2
0

toward baseline, whereas with the higher doses (0.30-0.90 μmoles), T_c entered a plateau phase before returning to normal. T_c and T_{sk} recovered concurrently in those rats in which intrathecal injection of 5-HT induced an initial tail skin vasoconstriction.

Changes in e.m.g. activity induced by intrathecal injections of 0.30 and 0.60 μmoles of 5-HT were assessed in five rats (Fig. 3). Both doses of 5-HT produced marked increases in shivering-like e.m.g. activity which were temporally related to the rising T_c. Moreover, the changes in e.m.g. recordings were accompanied by intense shivering-like activity which was visually evident in the hindquarters of the animal. For the 0.30 μmole dose, once the maximum T_c was attained, e.m.g. activity returned to preinjection levels or below. In contrast, at the 0.60 μmole dose, e.m.g. activity remained elevated through at least the first portion of the plateau phase of the T_c response and then fell to preinjection levels or below.

5-HT control solutions injected into the spinal subarachnoid space did not change T_c, T_{sk}, or e.m.g. activity. In addition, intrathecal injections of 5-HT did not cause obvious changes in behavior or motor function which might be related to thermoregulation.

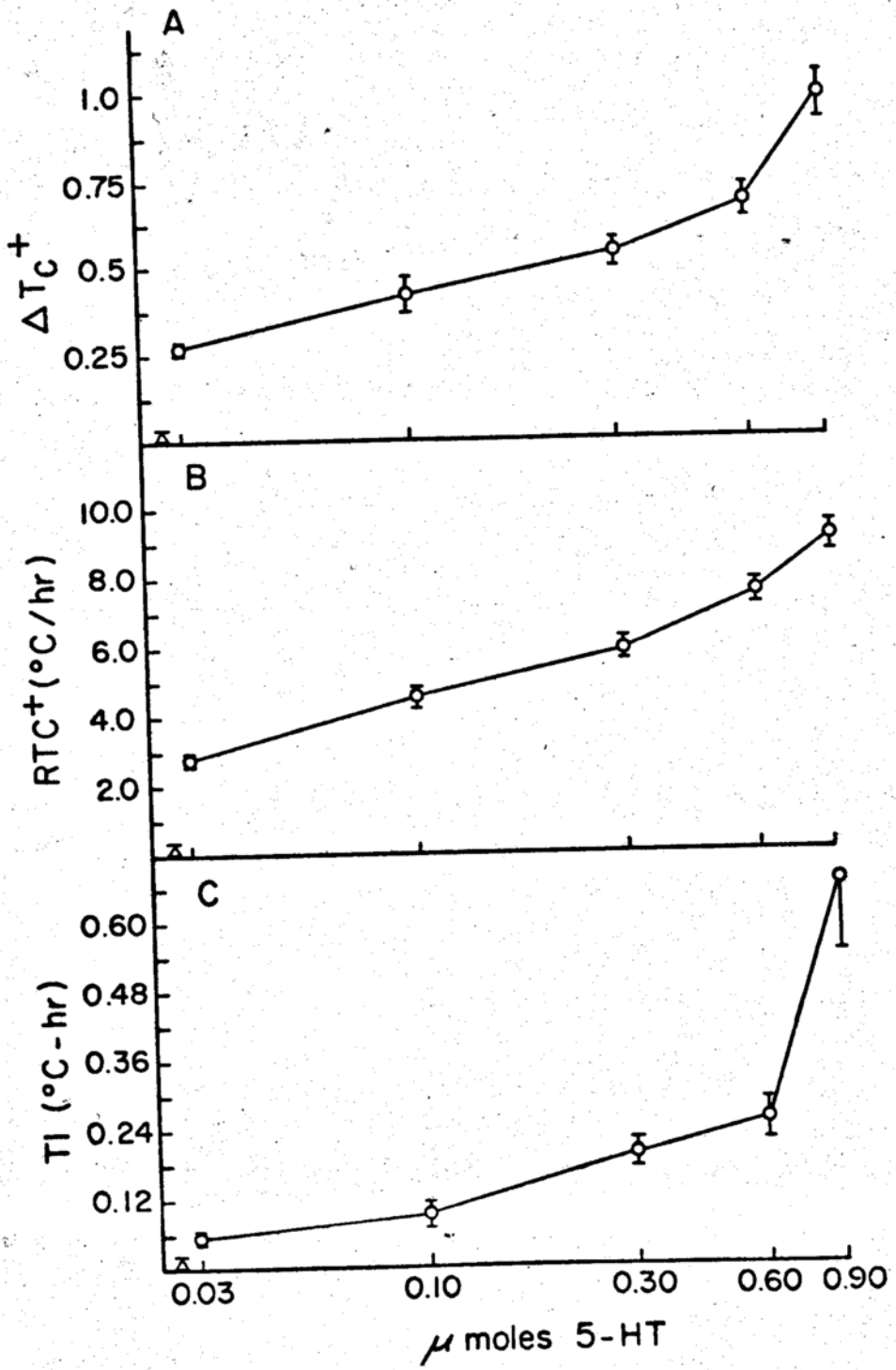
(iv) Dose-dependency of changes in thermoregulatory responses produced by intrathecal 5-HT

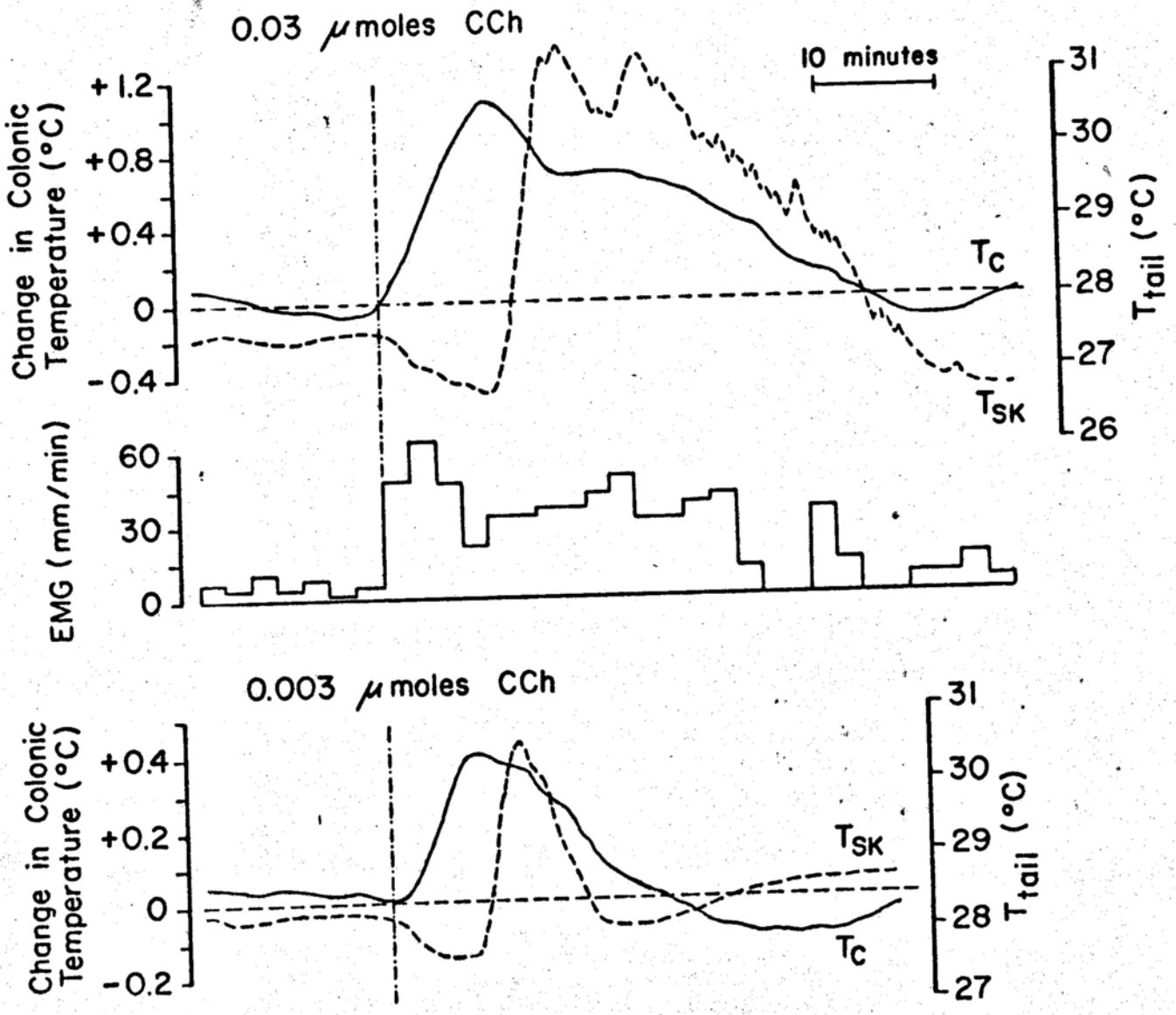
Intrathecal 5-HT injections produced dose-dependent changes in ΔT_c^+ (Fig. 4a), TI of the hyperthermia (Fig. 4b), and RTC^+ (Fig. 4c).

(v) General characteristics of the effects of intrathecal CCh on thermoregulation

Intrathecal administration of CCh produced an immediate, rapidly developing monophasic hyperthermia (Fig. 5; see Table 1 for doses). During the rising phase of the T_c response, T_{sk} fell slightly or remained constant. However, T_{sk} began to increase once the maximum T_c was attained. With the 0.01-0.06 µmole doses of CCh, T_c was maintained at a plateau. During this plateau phase, T_{sk} reached a maximum, and shortly thereafter T_c began to return to normal. Generally, T_{sk} remained elevated until T_c was at or near preinjection temperature. The smaller doses of CCh (0.003-0.006 µmoles) produced changes in T_c and T_{sk} which were qualitatively similar to the changes elicited by the higher doses. However, at these smaller doses, T_c and T_{sk} usually did not remain elevated but, rather, began to return to normal immediately after reaching a maximum.

The effects of two intrathecal doses of CCh (0.006 and


A
1.0
0.75
0.5
0.25
ΔT_c^+
B
10.0
8.0
6.0
4.0
2.0
RTC$^+$(°C/hr)
C
0.60
0.48
0.36
0.24
0.12
TI (°C-hr)
0.03
0.10
0.30
0.60
0.90
μ moles 5-HT


0.03 μ moles CCh
10 minutes
Change in Colonic Temperature (°C)
+1.2
+0.8
+0.4
0
-0.4
T_C
T_{SK}
31
30
29
28
27
26
T_{tail} (°C)
EMG (mm/min)
60
30
0
0.003 μ moles CCh
Change in Colonic Temperature (°C)
+0.4
+0.2
0
-0.2
T_{SK}
T_C
31
30
29
28
27
T_{tail} (°C)

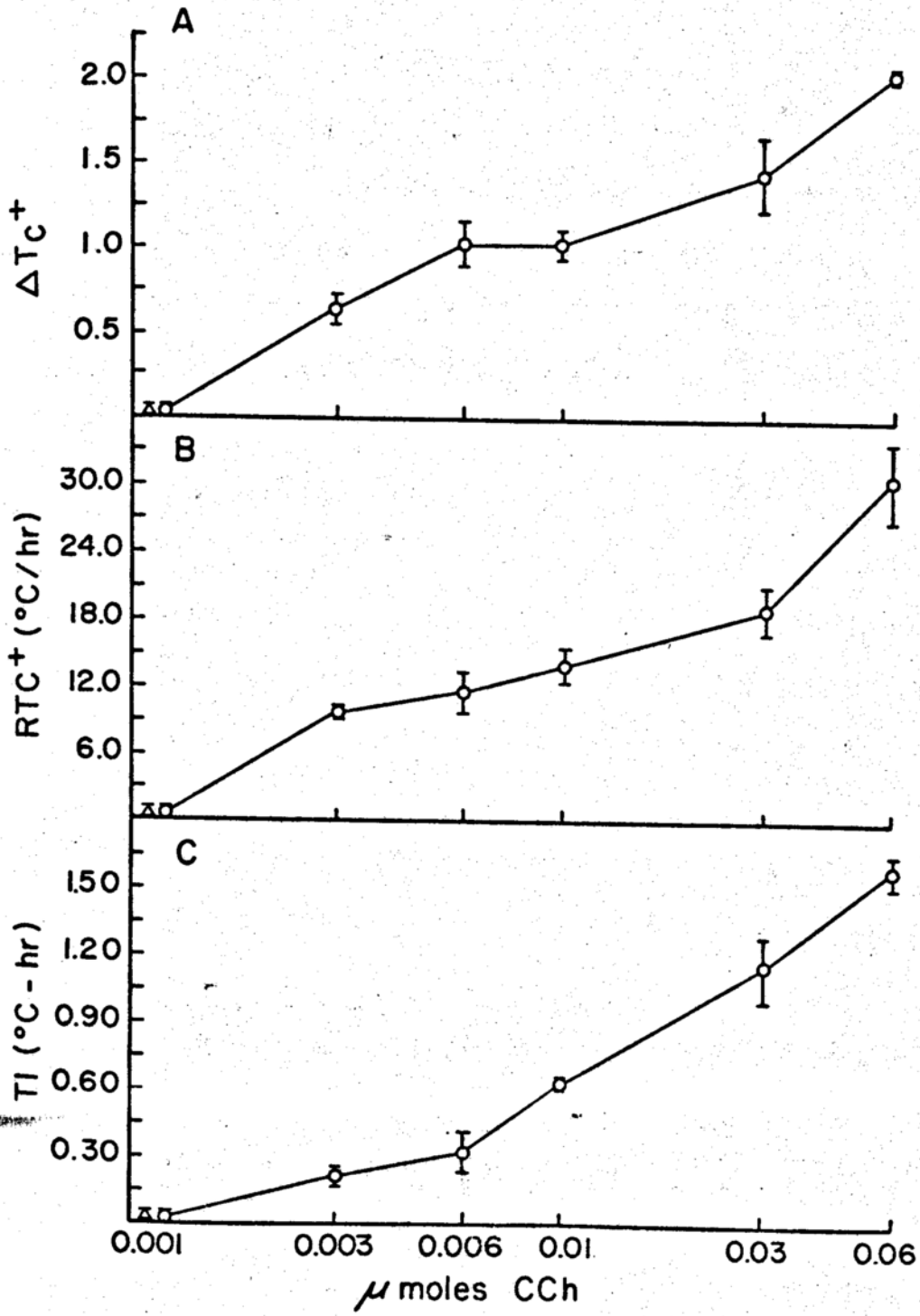
0.03 µmoles) on e.m.g. activity were examined in four rats. Both doses of CCh caused an immediate increase in shivering-like e.m.g. activity. The 0.006 µmole dose of CCh induced a brief rise in e.m.g. activity which was associated with the initial portion of the rising T_c. E.m.g. activity then returned quickly to preinjection levels or below. The larger dose of CCh (0.03 µmoles) produced an increase in e.m.g. activity which remained elevated during the plateau phase of the T_c response (Fig. 5). During the return of T_c toward normal, e.m.g. activity fell to preinjection levels or below.

Injections of CCh control solutions did not cause significant changes in thermoregulation. Nonthermoregulatory effects were not seen after any intrathecal dose of CCh.

(vi) Dose-dependency of changes in thermoregulatory responses produced by intrathecal CCh

Intrathecal injections of CCh promoted dose-related changes in ΔT_c^+ (Fig. 6a), Ti of the hyperthermia (Fig. 6b), and RTC^+ (Fig. 6c).

As indicated above, intrathecal CCh caused a delayed increase in T_{sk}. The ΔT_{sk}^+ produced by these injections was dose-dependent, with mean increases in T_{sk} ranging from 1.33°C (0.003 µmoles) to 5.92°C (0.06 µmoles) (data not shown).

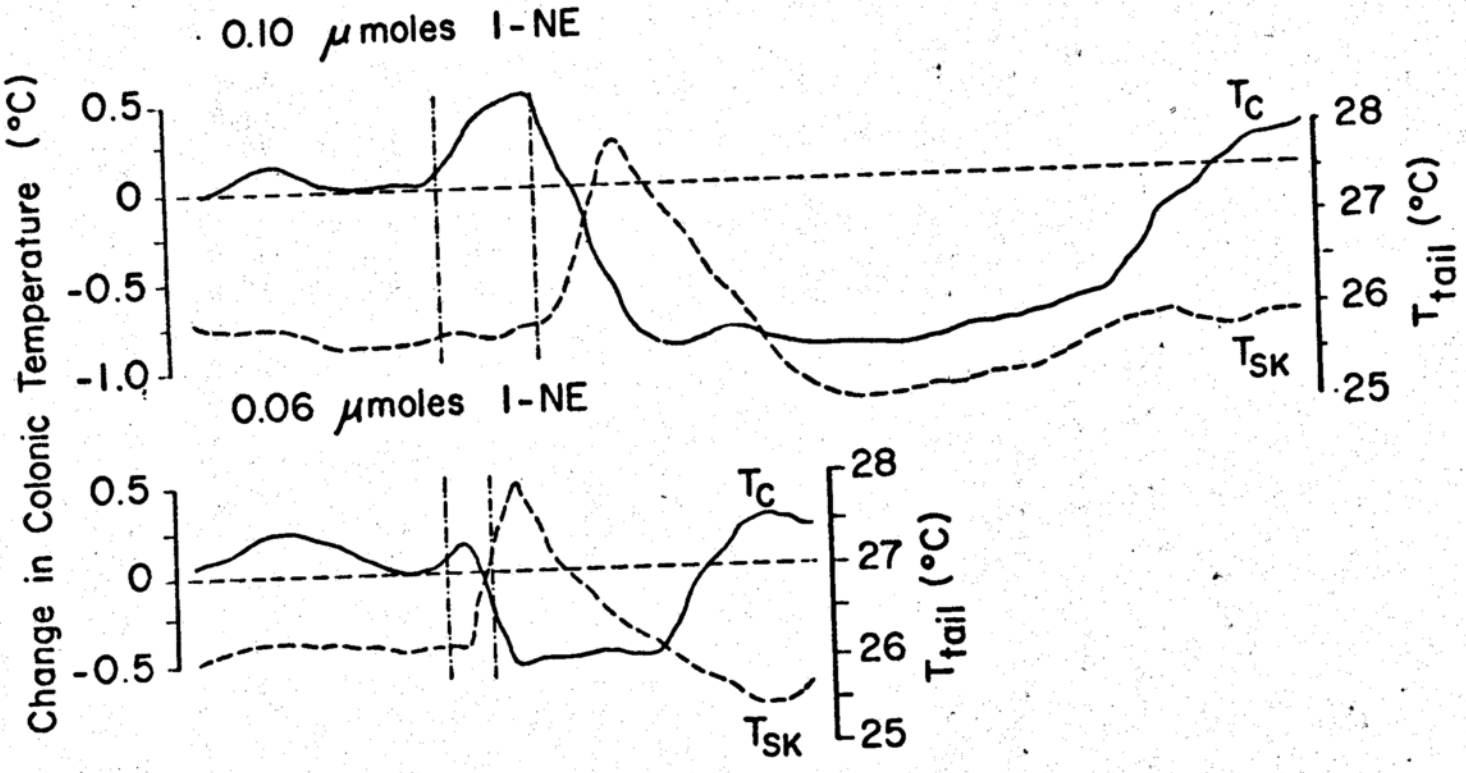

A
2.0
1.5
1.0
0.5
ΔTc+
B
30.0
24.0
18.0
12.0
6.0
RTC+ (°C/hr)
C
1.50
1.20
0.90
0.60
0.30
TI (°C - hr)
0.001
0.003
0.006
0.01
0.03
0.06
μ moles CCh

(vii) Effects of intravenous administration of NE, 5-HT, and CCh on thermoregulation

The possibility exists that some of the thermoregulatory effects of NE, 5-HT, and CCh injected intrathecally could be due to a direct action on peripheral thermoeffectors following leakage of these agents from their subarachnoid site of injection. To test this possibility, we determined the effects on thermoregulation of i.v. administration of two doses of NE, 5-HT, and CCh. The doses of drug given i.v. were comparable to those given intrathecally. Moreover, with the exception of one of the NE doses, drugs given i.v. were injected over 5, 10, and 15 minute periods to simulate several possible leakage rates of these agents from the spinal subarachnoid space.

No changes in thermoregulation were observed when 5-HT (0.30 and 0.90 µmoles) and CCh (0.006 and 0.03 µmoles) were injected i.v. over 5, 10, and 15 minute periods (N = 6).

In contrast, when NE was infused i.v., significant thermoregulatory responses were sometimes observed (Fig. 7). Infusion of 0.10 µmoles of NE over a 10 minute period produced hypothermia in four of six rats. Similar infusion of this dose of NE over 5 and 15 minute periods caused hypothermia in three of six rats and two of six rats, respectively. Regardless of the infusion rate, when hypothermia occurred following infusion of the 0.10 µmoles


0.10 μmoles 1-NE
Change in Colonic Temperature (°C)
0.5
0
-0.5
-1.0
T_C
T_{SK}
28
27
26
25
T_{tail} (°C)
0.06 μmoles 1-NE
0.5
0
-0.5
T_C
T_{SK}
28
27
26
25
T_{tail} (°C)

dose, T_c fell 0.80° (± 0.10°C). The hypothermic responses were usually preceded by a small rise in T_c which began during the infusion period. The changes in T_c were associated with a transient increase in T_{sk} which began during the 10 and 15 minute infusion periods and immediately after the 5 minute infusions of NE. Control solutions injected intravenously did not affect core or tail skin temperature.

In four of six rats, i.v. infusion of 0.06 μmoles of NE over a 5 minute period caused a brief hypothermia which was not preceded by hyperthermia. The average fall in T_c was 0.45° ± 0.05°C for those rats in which hypothermia developed. The fall was associated with a transient increase in T_{sk}, which began during the course of the infusion.

(viii) Injection of NE via intraperitoneal catheters

In the previous section (vii) results were discussed from experiments where NE was given i.v. at doses comparable to those used for intrathecal injections. These experiments were based on a "worst case" assumption that the entire dose of NE given intrathecally escaped into the systemic circulation. However, this probably was not the case, and consequently the thermoregulatory effects induced by i.v. NE at the doses used represent an overestimation of the thermoregulatory changes mediated by an action of NE in

the periphery following intrathecal injection of like doses. Thus, it was suggested that the absorption of NE from the peritoneal cavity into the systemic circulation might simulate the possible absorption of NE from the subarachnoid space into the periphery. Theoretically, with regards to entering the circulation, NE injected into the peritoneum should face barriers similar to those encountered by NE injected intrathecally (e.g., passage through cellular membranes). Any thermoregulatory changes induced by i.p. injection of NE might therefore be the best estimate of the changes in T_c and T_{sk} mediated solely by the peripheral actions of NE subsequent to leakage of this monoamine from the subarachnoid site of injection. Several doses of NE (0.06, 0.10, 0.30, and 0.60 µmoles) were injected into the peritoneal cavity of restrained rats (N = 5) via chronic indwelling catheters. Our results showed that the 0.30 and 0.60 µmole doses of NE produced a significant fall in T_c and a small, transient rise in T_{sk}, whereas the other doses of NE tested did not change either parameter. The fall in T_c produced by the 0.30 µmole dose of NE (0.46 $\pm$ 0.10°C), although smaller, was not statistically different from the hypothermia induced by the 0.60 µmole dose (0.76 $\pm$ 0.10°C). Similarly, the magnitude and duration of the tail temperature response for both doses of NE was not statistically different; each dose produced an

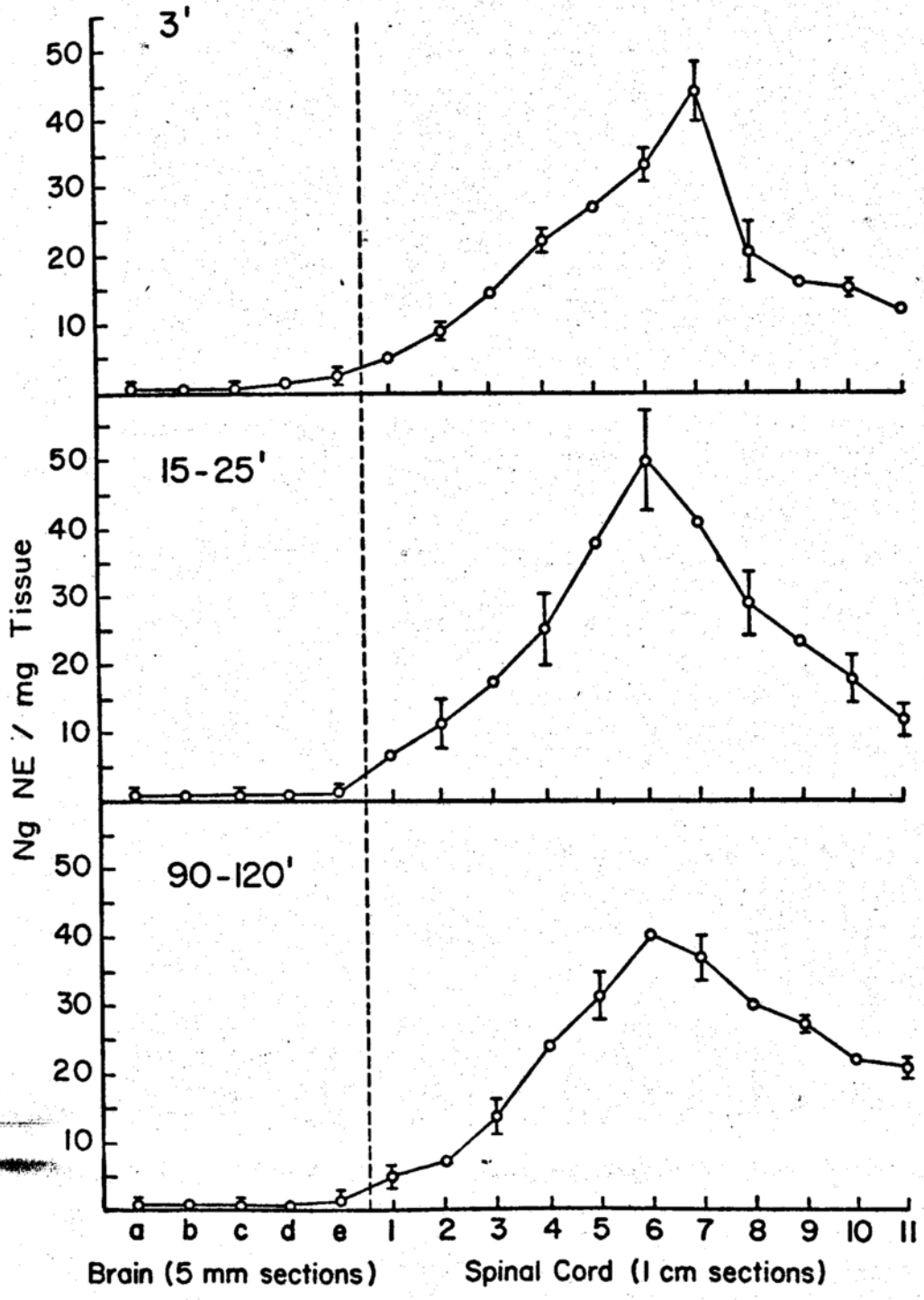
increase of about 3.75°C, which lasted from 30-45 minutes. The fall in T_c induced by i.p. injection of NE was often delayed by as much as 15 minutes, while the rise in T_{sk} began within 3 minutes of injection. Control injections of saline did not alter T_c or T_{sk}.

B. Phase II--Sites and Mechanism of Action for the Effects of Intrathecal NE on Thermoregulation

(i) Distribution of ^{3}H-NE following intrathecal injection

Spinal cord and brain. Figure 8 illustrates the distribution pattern of radiolabel along the neuroaxis at 3 minutes, 15-25, and 90-120 minutes after intrathecal injection of the ^{3}H-NE at 8.5 cm beyond the atlanto-occipital membrane. These time periods correspond to segments of the thermoregulatory response to intrathecal NE (0.30 µmoles) when T_c was rising during the hyperthermia, when T_c was falling during the hypothermia, and when T_c had stabilized at a level below normal, respectively. The proportion of the total injected activity present in the CNS (brain and spinal cord) was 36%, regardless of time of sacrifice.

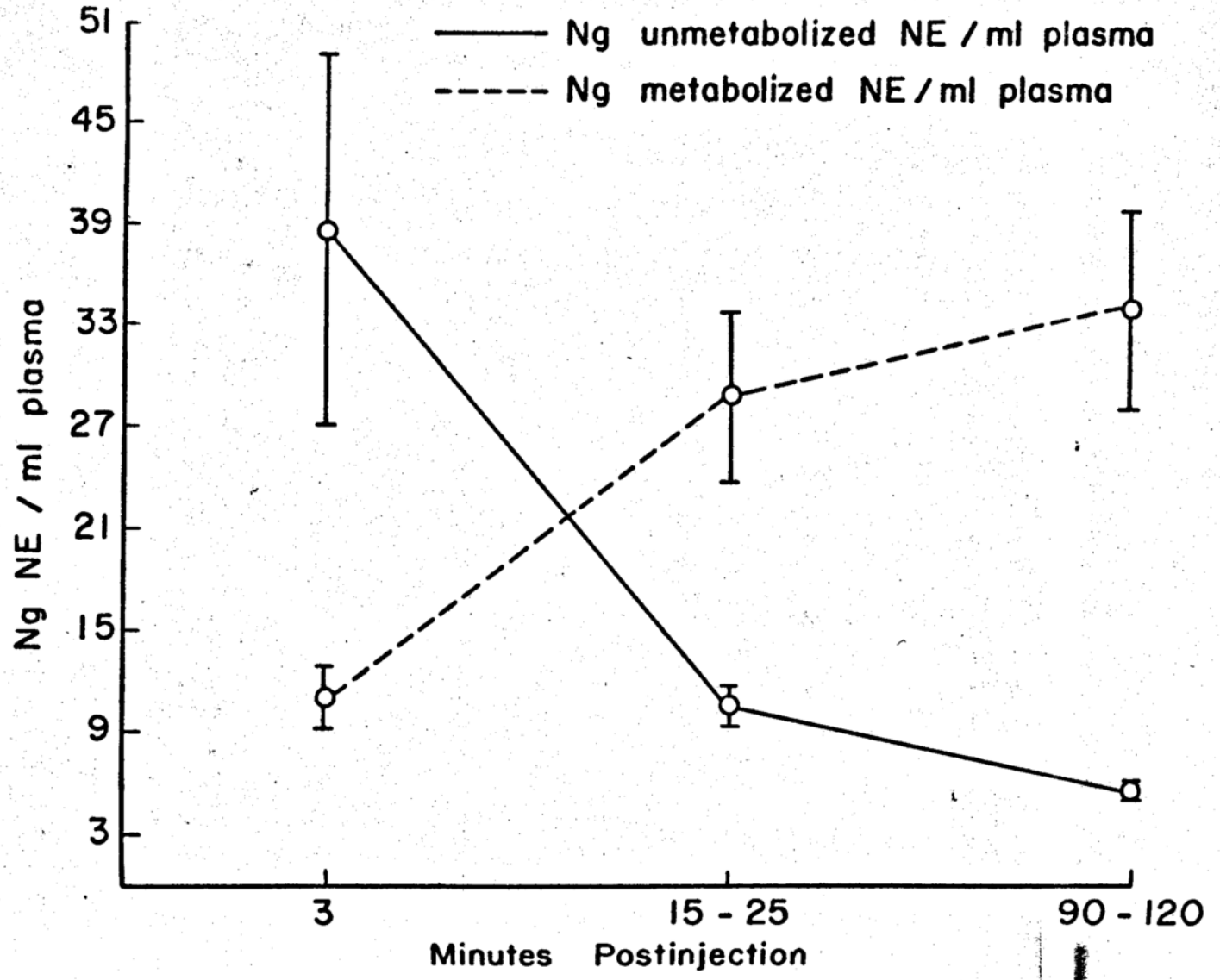
It is evident from Fig. 8 that the majority of ^{3}H-NE present in the CNS after an intrathecal injection was localized in the spinal cord regions for at least 2 hours after injection. Only small amounts of NE gained access to


3'
15-25'
90-120'
Ng NE / mg Tissue
50
40
30
20
10
a b c d e 1 2 3 4 5 6 7 8 9 10 11
Brain (5 mm sections)
Spinal Cord (1 cm sections)

supraspinal sites: at 3 minutes after injection, we found 3.00% of the total injected activity in the entire brain, whereas at the later time intervals, i.e., 15-25 and 90-120 minutes, we found 1.38% and 2.48%, respectively, of the injected activity in the brain. Autopsy revealed that the catheter tip was located near the lumbar enlargement in all rats. At the times tested, the highest tissue concentrations of radioactivity were found within 3-4 cm (C_8-S_4) on either side of the catheter tip.

Plasma. At 3 minutes after intrathecal injection of the 0.30 µmole dose of NE, a relatively large amount of unmetabolized NE (38.6 ± 11 ng/ml) was found in plasma (Fig. 9). At the later time periods examined, i.e., 15-25 and 90-120 minutes, progressively smaller amounts of unmetabolized NE (10 ± 1.2 ng, 5.8 ± 0.1 ng, respectively) were detected in the plasma. As the plasma level of unmetabolized NE decreased, the levels of metabolized NE increased (Fig. 9).

To ascertain whether the concentration of unlabeled NE in the injection mixture influenced the distribution of the radiolabel, two rats received intrathecal injections of 50 µCi of ^{3}H-NE only, and blood samples were taken at 3 minutes postinjection. The ratio of unmetabolized ^{3}H-NE to metabolites in the plasma of these two rats was similar to


Ng unmetabolized NE / ml plasma
Ng metabolized NE / ml plasma
Ng NE / ml plasma
51
45
39
33
27
21
15
9
3
3
15 - 25
90 - 120
Minutes Postinjection

that found in the plasma of rats which had received an intrathecal injection of a solution containing 0.30 µmoles unlabeled NE and 50 µCi ^{3}H-NE. It is therefore permissible to use the data obtained with 0.30 µmoles NE to compute the plasma concentrations of NE which were present after intrathecal injections of the other doses of NE employed. This information is presented in Table 2. In the table, the concentrations of unmetabolized NE shown were computed based on the assumption that the ratio of unmetabolized to metabolized NE at doses other than 0.30 µmoles was the same as that observed after injection of the 0.30 µmole dose.

(ii) <u>The effect of ganglionic blockade on the thermoregulatory response induced by intrathecal NE</u>

In these experiments, the thermoregulatory effects of NE (0.30 µmoles) injected intrathecally in rats pretreated with the ganglionic blocker mecamylamine were determined. The initial hyperthermia produced by the 0.30 µmole dose of NE could be due to a direct spinal effect (e.g., increasing sympathetic outflow) or to a peripheral action at receptors mediating non-shivering thermogenesis (NST) and vasomotor tone. If the hyperthermia were due to an action at spinal sites, then it should be prevented by ganglionic blockade. In preliminary studies, mecamylamine (10 mg/Kg, i.p.) caused a fall in T_c and a pronounced rise

Table 2

Extrapolated plasma concentrations of unmetabolized NE for each dose of NE given intrathecally

Time of sacrifice	μmoles NE injected intrathecally			
(minutes postinjection)	0.03	0.06	0.10	0.30
3	3.8[a]	7.5	12.8	38.6
15-25	1.0	2.0	3.4	10.0
90-120	0.5	1.0	1.7	5.8

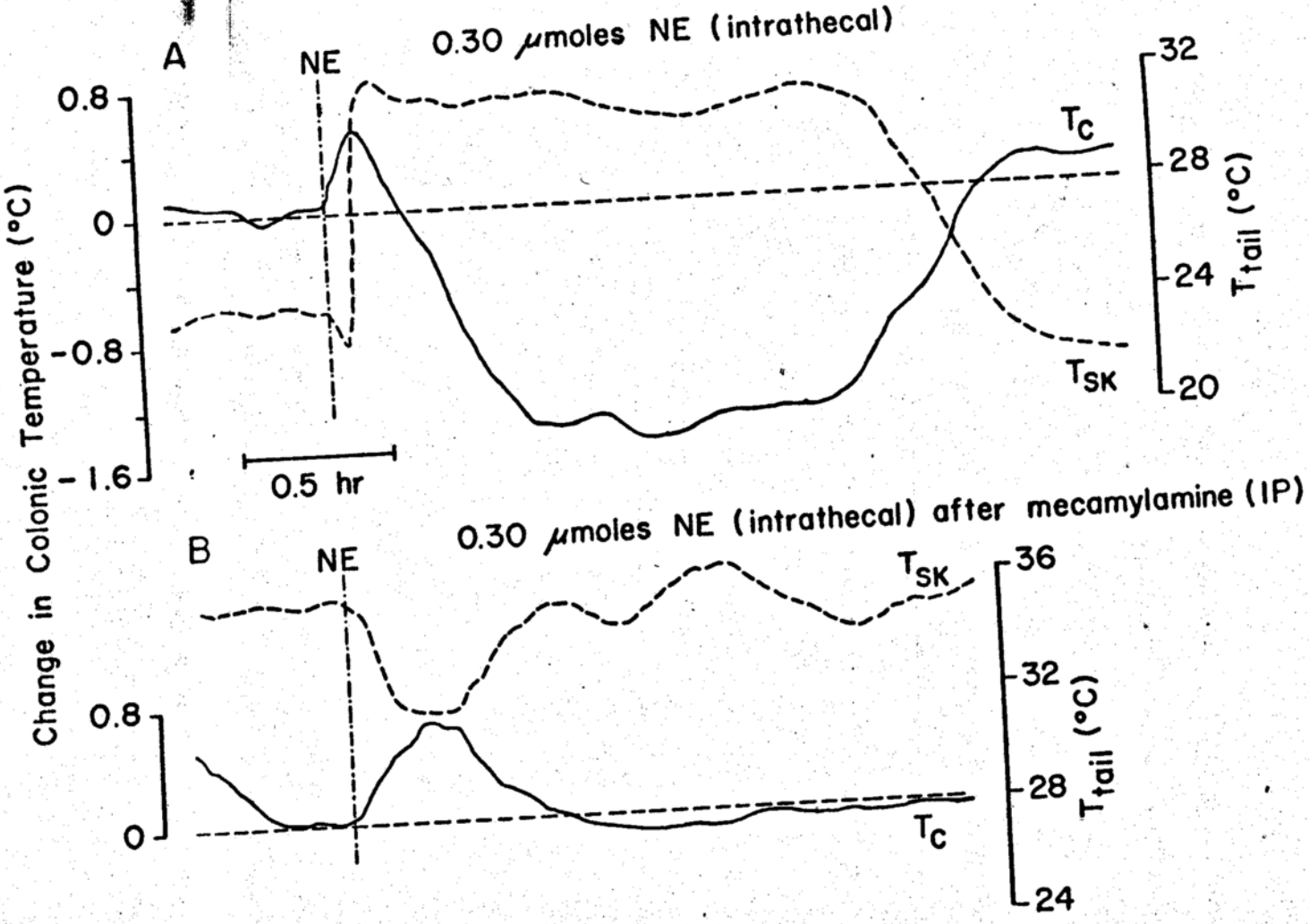
[a] ng NE/ml plasma

in T_{sk}. I.v. infusion of NE (5 µg/minute), given 45-60 minutes after mecamylamine, caused a brief increase in T_c and a fall in T_{sk}. After the NE infusion was stopped, T_c and T_{sk} rapidly returned to their pre-infusion levels. These results indicated that the mecamylamine pretreated rat could be a sensitive assay system for detecting physiologically significant quantities of exogenous NE in the systemic circulation.

Rats (N = 11) prepared with spinal catheters (8.5 cm) were pretreated with mecamylamine (10 mg/Kg, i.p.) and, 45-60 minutes later, 0.30 µmoles NE was injected intrathecally. NE injection caused a transient hyperthermia (0.65 $\pm$ 0.14°C) which was generally associated with a concomitant fall in T_{sk}. The time to reach the maximum T_c response was about 10 minutes. T_c and T_{sk} returned to the preinjection mecamylamine-induced temperatures within 30 minutes after intrathecal NE administration. The effects in one rat of intrathecal injection of NE with and without mecamylamine pretreatment are illustrated in Fig. 10.

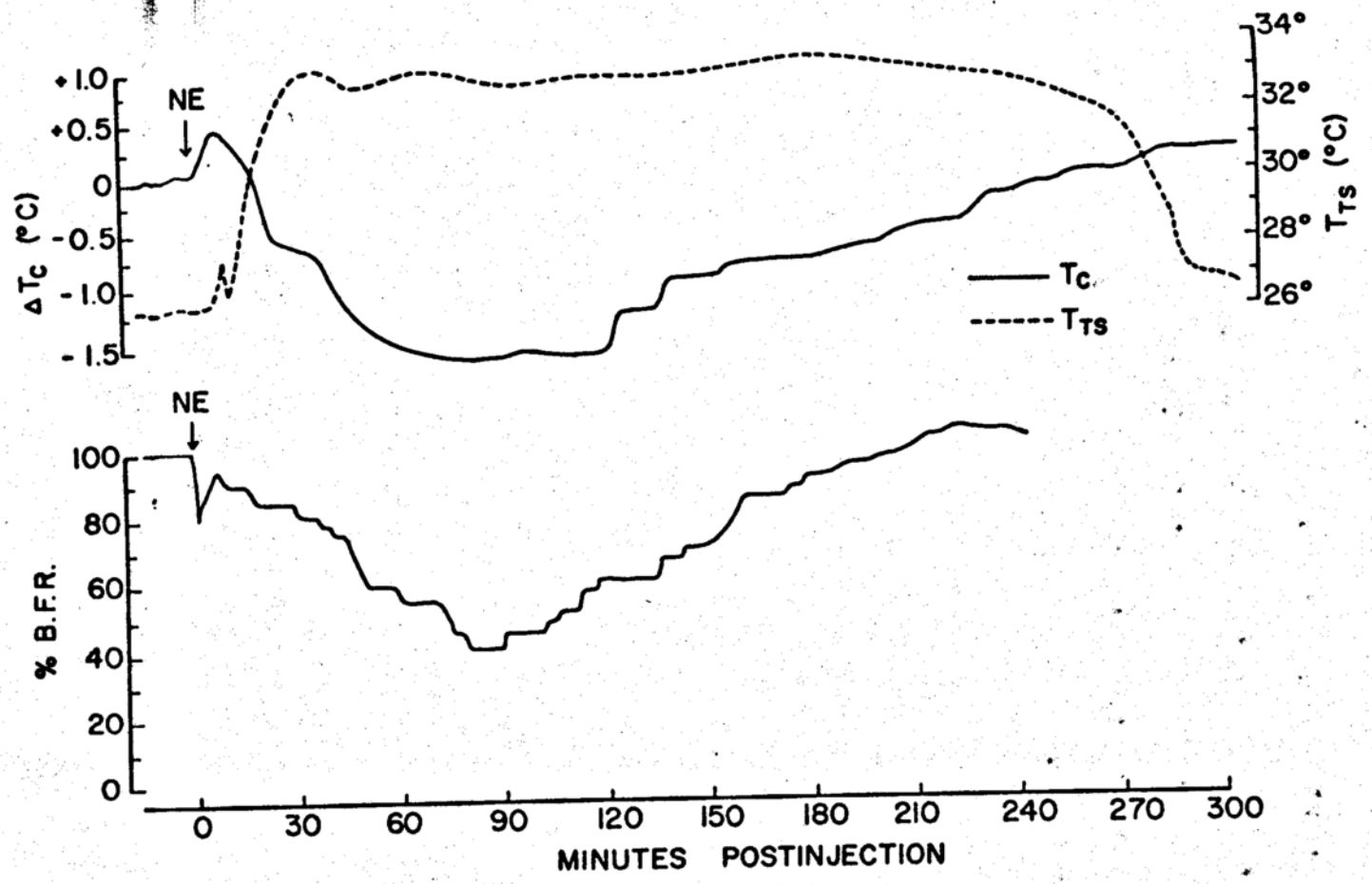
(iii) <u>The effect of intrathecal NE and clonidine on neural activity recorded from the lumbar sympathetic chain</u>

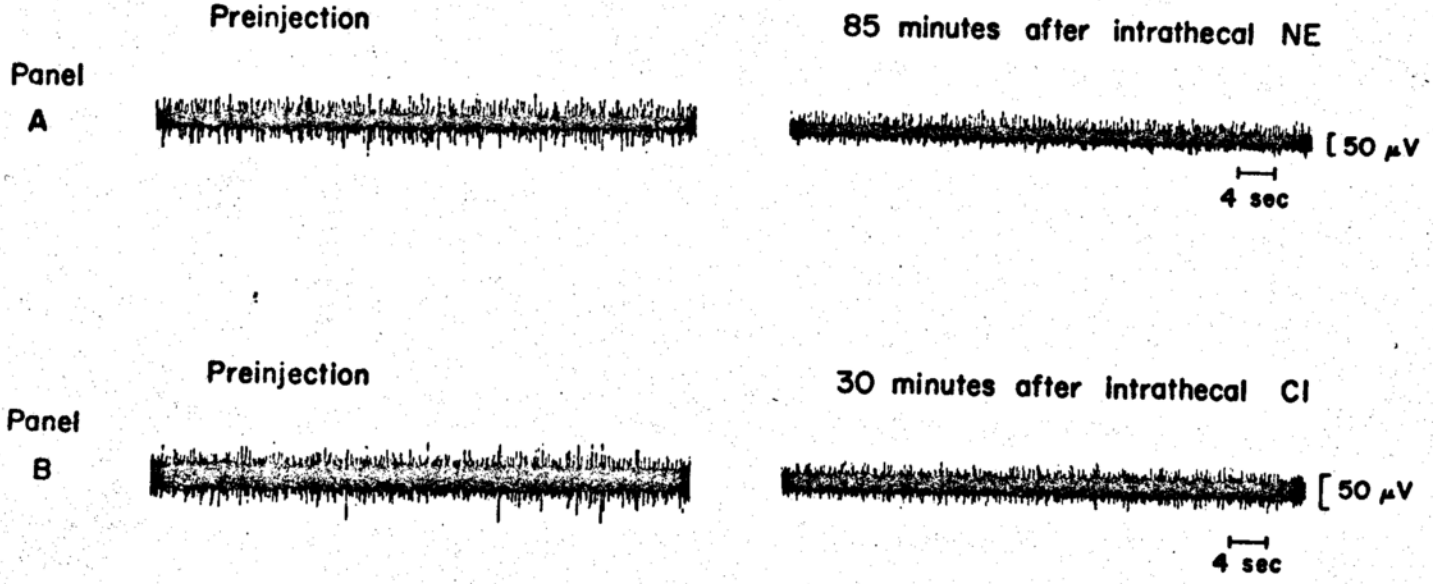
These experiments were designed to determine whether NE and clonidine, injected intrathecally, could

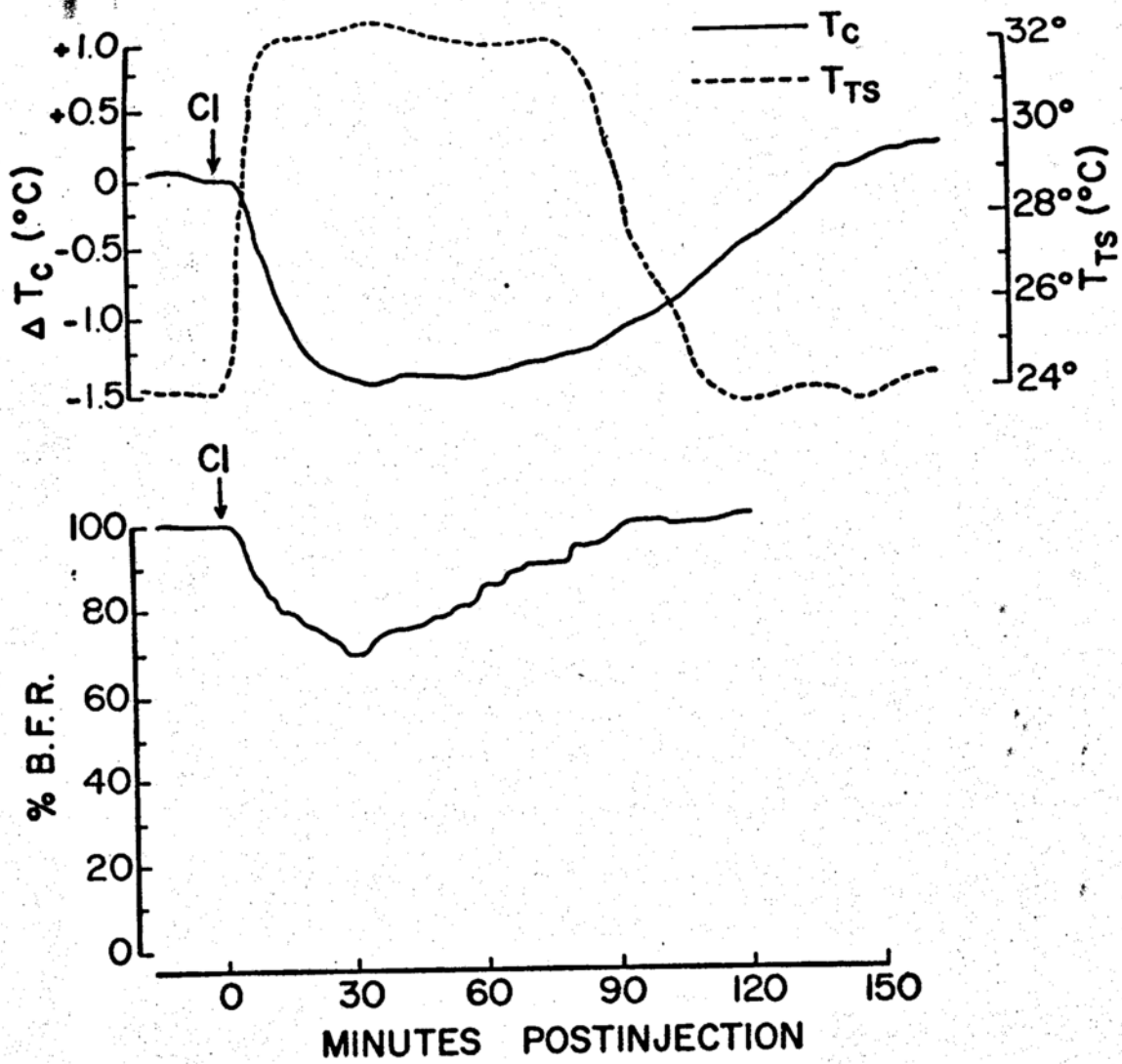

A
NE
0.30 μmoles NE (intrathecal)
0.8
0
-0.8
-1.6
Change in Colonic Temperature (°C)
0.5 hr
T_C
T_{SK}
32
28
24
20
T_{tail} (°C)
B
NE
0.30 μmoles NE (intrathecal) after mecamylamine (IP)
T_{SK}
T_C
0.8
0
36
32
28
24
T_{tail} (°C)

decrease efferent sympathetic activity. Intrathecal injections of control solutions for NE and clonidine did not alter lumbar chain activity. Intrathecal injection of NE (0.30 μmoles) produced an immediate transient decrease in lumbar firing rate. This initial effect was followed by a more gradually developing, prolonged inhibition of sympathetic activity. During this latter phase of the response, lumbar chain activity was reduced to 51 ± 45% of baseline. Figure 11 shows this effect of intrathecal NE in one rat and also illustrates the changes in T_c and T_{sk} induced by intrathecal NE given two days earlier in the same rat. Pre- and post-injection samples of lumbar firing activity are presented in Fig. 12a. Generally, the maximum decrease in neural firing rate occurred approximately one hour after intrathecal injection and remained at this level of depression for 10-15 minutes before returning gradually to the preinjection level. Full recovery of neural firing rate usually required two to three hours. Intravenous infusions of NE did not cause depressions of sympathetic firing rates which extended beyond the time of infusion.

Intrathecal injections of clonidine (0.035 μmoles) in two rats produced monophasic depressions of lumbar sympathetic firing rate (Figs. 13 and 12b). These drug-induced responses reached their maximum, i.e., firing rate reduced to 70% and 72% of baseline, within 30 minutes of injection.


+1.0
+0.5
0
-0.5
-1.0
-1.5
ΔTc (°C)
NE
34°
32°
30°
28°
26°
TTS (°C)
Tc
TTS
NE
100
80
60
40
20
0
% B.F.R.
0
30
60
90
120
150
180
210
240
270
300
MINUTES POSTINJECTION


Preinjection
85 minutes after intrathecal NE
Panel A
50 μV
4 sec
Preinjection
30 minutes after intrathecal CI
Panel B
50 μV
4 sec


T_C
T_{TS}
+1.0
+0.5
0
-0.5
-1.0
-1.5
ΔT_C (°C)
CI
32°
30°
28°
26°
24°
T_{TS} (°C)
CI
100
80
60
40
20
0
% B.F.R.
0
30
60
90
120
150
MINUTES POSTINJECTION

Neural firing rates returned from drug-induced maximum effects to preinjection levels over the next 45-60 minutes.

(iv) The effects of varying the spinal catheter length on the thermoregulatory response to intrathecal NE

To determine whether the thermoregulatory effects of intrathecal NE were altered by changing the distribution of NE along the cord, rats were prepared with spinal catheters extending either 4 cm or 12 cm beyond the atlanto-occipital membrane. The results from these rats were compared to previous data from rats with 8.5 cm catheters. All rats received a 0.30 µmole dose of NE dissolved in 4 µl of ACSF. These injections produced modified thermoregulatory responses compared to the changes in T_c and T_{sk} induced by intrathecal injections of NE (0.30 µmoles) in rats with 8.5 cm catheters. Figures 14a, 10a, and 14c illustrate the thermoregulatory changes induced by intrathecal injection of NE in rats prepared with 4 cm, 8.5 cm, or 12 cm spinal catheters, respectively. Table 3 provides a quantitative comparison of the thermoregulatory effects of NE administered intrathecally in the above groups of rats. The results show that the NE-induced hyperthermia in rats with 12 cm catheters was significantly larger in magnitude and longer in duration than the hyperthermia observed in rats with 4 cm catheters. However, with

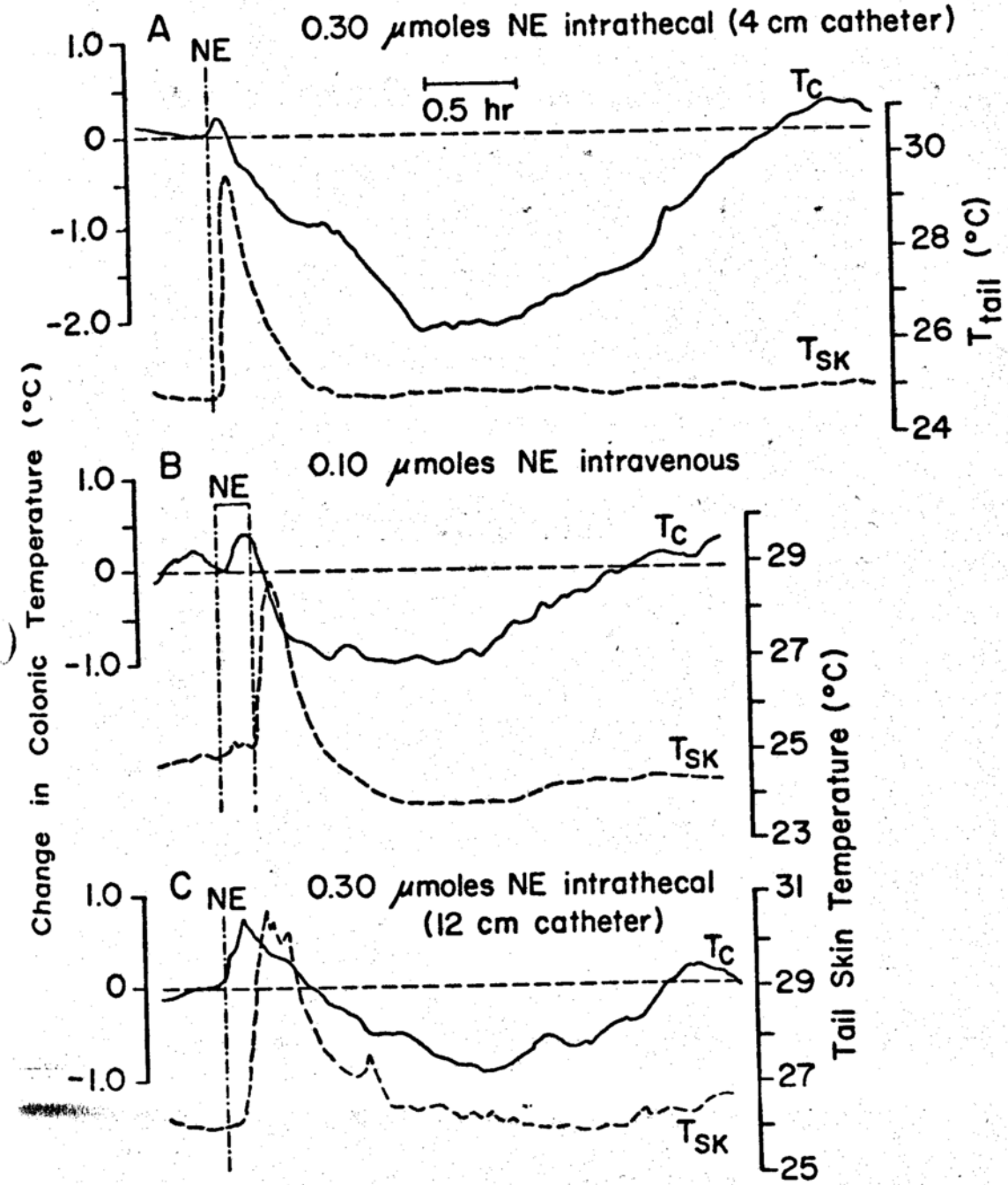

A
NE
0.30 μmoles NE intrathecal (4 cm catheter)
0.5 hr
1.0
0
-1.0
-2.0
T_C
T_{SK}
30
28
26
24
T_{tail} (°C)
B
NE
0.10 μmoles NE intravenous
1.0
0
-1.0
T_C
T_{SK}
29
27
25
23
Change in Colonic Temperature (°C)
C
NE
0.30 μmoles NE intrathecal
(12 cm catheter)
1.0
0
-1.0
T_C
T_{SK}
31
29
27
25
Tail Skin Temperature (°C)

Table 3

Comparisons of the thermoregulatory effects of NE injected intrathecally in rats prepared with either 4 cm (N = 9), 8.5 cm (N = 6), or 12 cm (N = 8) spinal catheters.[a]

	Catheter Length		
Parameter	4 cm	8.5 cm	12 cm
ΔT_c^+ (°C)	0.16 ± 0.07[b]	0.41 ± 0.13	0.73 ± 0.11†
Duration of T_c^+ effect (hours)	0.16 ± 0.07	0.28 ± 0.07	0.49 ± 0.12†
ΔT_c^- (°C)	1.80 ± 0.11	1.87 ± 0.27	0.73 ± 0.16*
Duration of T_c^- effect (hours)	3.10 ± 0.18	3.25 ± 0.07	1.75 ± 0.42*
ΔT_{sk}^+ (°C)	2.04 ± 0.04≠	6.92 ± 0.49	1.88 ± 0.46≠
Duration of T_{sk}^+ effect (hours)	0.44 ± 0.06≠	4.0 ± 0.41	0.71 ± 0.21≠

[a] † = significantly different from 4 cm

* = significantly different from 8.5 and 4 cm

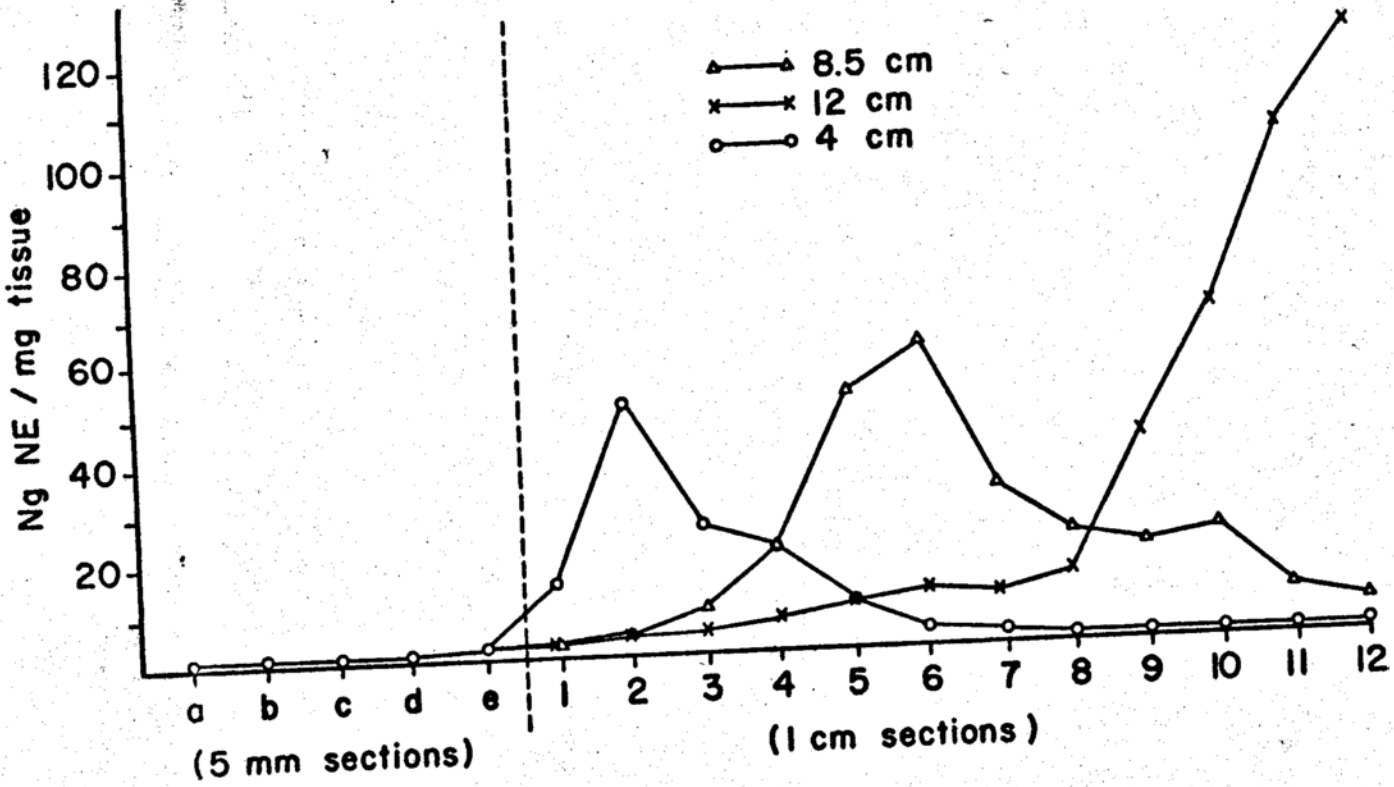
≠ = significantly different from 8.5 cm

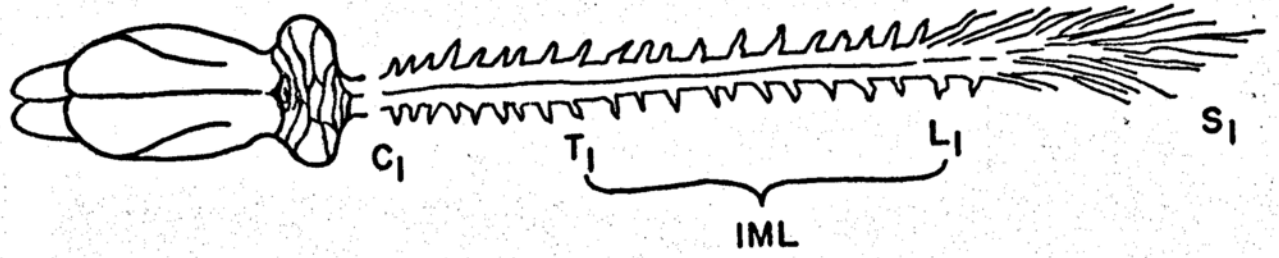
All other comparisons between means were not statistically different ($P < 0.05$).

[b] All table values are means ± SE

respect to magnitude and duration, the hyperthermia associated with the above two groups of rats (4 cm, 12 cm) was not statistically different from the rise in T_c induced by intrathecal injections of NE in rats with 8.5 cm catheters. The hypothermic response of rats with 12 cm catheters was significantly smaller in magnitude and duration than the hypothermia observed in the other two groups of rats (4 cm, 8.5 cm). Our results also indicated that the rise in T_{sk} seen in rats with 4 cm and 12 cm catheters was much shorter in duration and smaller in magnitude in comparison to the NE-induced tail temperature response of the rats with 8.5 cm long catheters.

In five animals, studies of the distribution of ^{3}H-NE (50 µCi/4 µl) along the neuroaxis of rats with 4 cm and 12 cm catheters showed that less than 2% of the injected activity was found in supraspinal areas of each of these rats. Moreover, at 30 minutes after intrathecal ^{3}H-NE injection in rats with 4 cm catheters, most of the radioactivity recovered from the CNS was localized to the cervical and upper thoracic areas of the spinal cord, whereas in rats with 12 cm catheters, the radioactivity was concentrated in spinal regions caudad to T_{13} (Fig. 15).


8.5 cm
12 cm
4 cm
Ng NE / mg tissue
120
100
80
60
40
20
a b c d e 1 2 3 4 5 6 7 8 9 10 11 12
(5 mm sections)
(1 cm sections)


C1
T1
L1
S1
IML

V. DISCUSSION

A. Phase I--the Effects of Intrathecal Injections of NE, Clonidine, 5-HT, and CCh on Thermoregulation

(i) Effects on thermoregulatory function of intrathecal NE, 5-HT, and CCh

In theory, an agent injected into the spinal subarachnoid space could alter body temperature by acting on either the afferent or efferent aspect of the thermoregulatory system. An action on spinally situated thermodetectors or upon the rostrad transmission of impulses from thermodetectors in the skin or body core could introduce a false or a biased input to the system. The functional consequence of a chemically mediated biasing of thermal afferent pathways would be to change the apparent level of the set-point for thermoregulation. Rudy and Yaksh (1977) proposed that such a mechanism was responsible for the apparent increase in the set-point produced by intrathecal injection of morphine in the rat. An agent which altered body temperature through an action on the efferent side of the system could do so by directly activating or inhibiting spinal pathways controlling peripheral thermoeffectors or by modulating the level of endogenous activity in these pathways. The results, in contrast to an effect on afferent systems, would be a relatively inflexible and possibly

uncoordinated pattern of changes in effector activities.

In the present experiments, injection of NE (0.03-0.30 μmoles) and clonidine (0.0175-0.070 μmoles) into the rat spinal subarachnoid space produced a dose-dependent fall in T_c and a rise in T_{sk}. The hypothermia evoked by the highest dose of NE (0.30 μmoles) was preceded by a short hyperthermia. In a significant number of sessions (10 of 24), the fall in T_c induced by NE began well in advance of the tail temperature response. Moreover, the hypothermia was not associated with an inhibition of shivering or thermoregulatory muscle tonus, since e.m.g. activity remained at the baseline level or increased during the fall in T_c. This lack of coordination among thermoeffectors is not consistent with a NE-induced decrease in the set-point level. Instead, the effects of NE may have been the result of an action upon individual sympathetic effector systems such as those involved in the control of vasomotor tone and NST (vide infra).

Intrathecal injection of 5-HT caused an immediate, rapidly developing hyperthermia associated with coordinated changes in shivering and tail vasomotor tone. The concerted change in thermoeffector activities argues in favor of the hypothesis that the hyperthermic response represents a 5-HT-induced increase in the set-point mediated by an action within thermal afferent pathways. However, it

must be stressed that simple observation of the pattern of effector activities at one ambient temperature, as was the situation in these experiments, is insufficient to differentiate a coordinated change in effector activities due to an action on afferent systems from a coordinated change resulting from the simultaneous, but independent, activation of several effector outflow pathways. Additional studies, e.g., the injection of the agonist at different ambient temperatures, would be required to distinguish between these two mechanisms of action.

The effects of intrathecal CCh on thermoregulation are complex. CCh produced an immediate, rapidly developing hyperthermia. As T_c rose, shivering-like activity increased and T_{sk} decreased. Once the maximum change in T_c had been reached, T_{sk} increased rapidly. At the 0.01-0.06 µmole doses of intrathecal CCh, the plateau phase of the T_c response was associated with continued shivering-like activity and tail skin vasodilation. The thermoeffector coordination observed while T_c was rising suggests that the CCh response may have been mediated by an action on afferent pathways. However, the simultaneous presence of shivering and vasodilatation during the plateau phase suggests that CCh concomitantly activated thermogenic and heat dissipation mechanisms or that the vasodilatation was compensatory.

(ii) Receptor specificity of the effects of intrathecal NE, 5-HT, and CCh

At the outset, it should be stated that it remains to be proven that intrathecally injected NE, 5-HT, and CCh produced thermoregulatory effects by acting directly on agonist-specific pre- or post-junctional receptors. However, several lines of evidence argue in favor of specificity of action: 1) The effects were not due to artifacts related to the volume of injection or the pH, osmolality, or ionic content of the injectates, because injections controlling for these factors had no effect on thermoregulation; 2) Agonist-specific receptors with which the injected agents could have interacted are present in the spinal cord. Serotonergic receptors (Nelson et al., 1980), alpha and beta noradrenergic receptors (Alexander et al., 1975; Young and Kuhar, 1979), and muscarinic and nicotinic cholinergic receptors (Myslinski and Randic, 1977; Kayaalp and Neff, 1980) have been found in several regions of the spinal cord grey matter; 3) The effects of each of the injected agents on most of the thermoregulatory parameters measured was clearly dose-dependent. In this regard, it is of particular import that the RTC parameter was in each case dose related. ΔT_c and, especially TI, are strongly influenced by the length of time that the drug remains in the vicinity of the injection site, and one

might expect these parameters to be dose related purely on the basis of the increased duration of action associated with increased dosage. The RTC parameter, on the other hand, reflects more accurately the relationship between response and drug concentration present in the biophase during the first few moments after injection. The presence of a regular relationship between dose and thermoregulatory response does not prove that the injected agents acted on specific receptors. However, the absence of a highly irregular or bell-shaped dose effect relationship is significant, for these latter functional relationships have been considered indicative of nonspecific activity (Beckman, 1970; Bruinvels, 1970); 4) Intrathecal injection of small doses (0.0175-0.070 μmoles) of the α-adrenergic agonist clonidine produced dose-dependent changes in thermoregulation similar to those induced by intrathecal NE. Moreover, intrathecal injections of a large dose (0.30 μmoles) of d-NE, a NE enantiomer which is a weak agonist at noradrenergic receptors, caused little or no thermoregulatory effect; 5) In preliminary studies employing specific receptor antagonists, we have found that intrathecal pretreatment with atropine greatly attenuated the hyperthermic effect of CCh injected intrathecally.

(iii) The spinal cord as the site of action for NE, 5-HT, and CCh injected intrathecally

A drug injected into the lumbar subarachnoid space might be carried in the CSF to supraspinal sites where drug-induced changes in thermoregulation could be mediated. However, previous work (Yaksh and Rudy, 1976; Yeung and Rudy, 1980), and that discussed in section B, has shown that substances of widely varying molecular weight and lipid solubility (NE, urea, morphine, naloxone) did not reach the supraspinal structures in appreciable concentrations within the first two hours after lumbar intrathecal injection. As each of the substances employed in the present study elicited thermoregulatory effects within a few minutes after injection, an involvement of supraspinal structures in the mediation of these responses seems highly unlikely. For 5-HT and CCh, an additional argument against an action at supraspinal sites is the disparity in thermoregulatory effects evoked by these agents applied intrathecally and supraspinally. Intrathecal injections of these agents evoked hyperthermia, whereas 5-HT and CCH IVT or IC have generally been found to produce a fall in T_c (Clark and Clark, 1980a, b).

An important consideration in any study of thermoregulation involving the central administration of a drug is the possibility that the injected drug produced its effects

on temperature regulation by directly stimulating peripheral thermoeffectors subsequent to leakage of the agent from its central site of injection. The results from the present study show that the thermoregulatory effects of intrathecal 5-HT and CCh are not produced by leakage to a peripheral site or sites of action; i.v. injections of large doses of these agents did not alter T_c or T_{sk}.

In contrast, i.v. injection of the 0.10 µmole dose of NE caused an initial hyperthermia followed by a fall in T_c associated with a brief rise in T_{sk}. I.v. injections of the 0.06 µmole dose, and i.p. injections of the 0.30 and 0.60 µmole dose of NE caused a monophasic hypothermia and a transient tail skin vasodilation. Why hyperthermia was associated with only the highest dose of NE (0.10 µmoles) given i.v. remains to be determined. It could be suggested that following i.p. injections of NE and i.v. infusion of the 0.06 µmole dose, the blood levels of unmetabolized NE attained were too low to produce hyperthermia but may have been large enough to induce a fall in T_c (vide infra). Regardless, the above data suggest that the thermoregulatory effects elicited by intrathecal NE might have been mediated, at least in part, by an action within the periphery rather than upon the spinal cord.

Physiologically significant amounts of NE appear in the peripheral circulation following IVT, IC, and IH

injections (Mayer et al., 1960; Glowinski et al., 1965; Struyker-Boudier et al., 1974; Buccafusco and Brezenoff, 1977), and it thus seems likely that NE injected into the spinal subarachnoid space also escapes into the circulation. Moreover, the hypothermic effect of i.v. NE reported in this study is consistent with previous reports which have suggested that the hypothermia produced by parenteral injections of other alpha-sympathomimetics (e.g., clonidine, naphazoline) is either partially (Tsoucaris-Kupfer and Schmidt, 1972) or totally (Lomax and Foster, 1969) mediated by actions at peripheral sites. How this hypothermia might be produced has not been determined, but it has been suggested that the sympathomimetics might interfere with carbohydrate metabolism (Bain and Kohlenbrenner, 1950). In addition, NE has been found to block ganglionic transmission (DeGroate and Volle, 1966), which could produce vasodilation and hypothermia.

Thus it appears from the above discussion that the thermoregulatory effects of intrathecal NE could be due to an action of this monoamine at peripheral sites. However, based solely on evidence from Phase I experiments it is reasonable to conclude tentatively that the hypothermia and tail skin vasodilation elicited by NE, especially by the lower doses, were mediated primarily by an action on the spinal cord. In the first place, intrathecal injections of

NE consistently produced hypothermia, whereas i.v. injections, although they reliably evoked transient vasodilation, caused a fall in T_c in only 50% of the rats tested. Secondly, the tail skin vasodilation which occurred after an intrathecal dose of NE was always greater in magnitude and much longer in duration than that which occurred after a comparable dose of NE given i.v. Thirdly, the magnitude of the hypothermic response elicited by i.v. doses of NE was significantly less than that produced by the same dose given intrathecally. This was true even when rats in which i.v. NE produced no fall in body temperature were excluded from the analysis. The importance of the second and third points made above is enhanced by the likelihood that not all of the intrathecally injected NE escaped into the circulation. Glowinski et al. (1965) and others (vide supra) reported that, within the first few hours after injection, only 50-60% of NE administered into the supraspinal CSF escaped into the periphery. Thus, a comparison of the thermoregulatory effects of equal doses of NE given intrathecally and i.v. very likely exaggerates the putative peripheral component of the effect of intrathecal NE.

The largest intrathecal dose of NE examined in the present experiments (0.30 µmoles) produced a hypothermia which was preceded by a brief rise in T_c. This initial hyperthermia was not produced by any of the lower

intrathecal doses of NE tested and may be a product of a nonspecific, high-dose effect on the spinal cord. However, a similar initial hyperthermic effect was seen in rats injected i.v. with 0.10 μmoles NE (the highest i.v. dose tested). It is possible, therefore, that this effect of NE is peripherally mediated, perhaps by activation of the noradrenergic receptors involved in the control of NST. In section B, results will be discussed from Phase II experiments which examined the efflux of NE into the periphery following intrathecal injection and the possible thermoregulatory consequences of exogenous unmetabolized NE in the systemic circulation.

(iv) Potential spinal sites of action for NE, 5-HT, and CCh

In the present investigation, the agonists studied were injected into the CSF-filled space surrounding the lumbar spinal cord. Based on the distribution of other substances injected into the rat lumbar subarachnoid space (Yaksh and Rudy, 1976; Yeung and Rudy, 1980), it seems likely that NE, 5-HT, and CCh produced thermoregulatory effects by acting at sites located in the caudal two-thirds of the spinal cord. In view of the rapid onset of thermoregulatory effects after intrathecal injection, it also seems likely that the sites of action are situated near the

cord surface rather than deep within the spinal grey matter. Among the many possible neuroanatomical substrates which might have been reached by the injected agents, four seem worthwhile discussing at the present time: 1) The dorsal horn; 2) Spinal thermodetector units; 3) The intermediolateral cell column; and 4) The ventral horn.

Primary afferent neurons synapse with second order neurson (Angel, 1977) in the marginal layer (lamina I) and substantia gelatinosa (laminae II and III) of the dorsal horn grey matter. These secondary neurons project to ascending spinal pathways, such as the spinothalamic and spinoreticular tracts, which convey sensory information (i.e., pain and temperature) to supraspinal structures (Angel, 1977). Research has suggested that the transmission of sensory information from primary to secondary neurons in the dorsal horn is modulated by descending bulbospinal input (Wall, 1967). Bulbospinal noradrenergic and serotonergic fibers terminate in the dorsal horn (see Introduction), and several investigators have suggested that these descending pathways might modulate sensory transmission, since intrathecal injections of NE and 5-HT have been shown to produce analgesia (Yaksh and Wilson, 1979; Reddy et al., 1980). Moreover, the activity of high threshold and wide dynamic range spinothalamic tract neurons, which transmit nociceptive and thermal information

to supraspinal sites, was depressed by microiontophoretic application of 5-HT and NE (Headley et al., 1978; Satoh et al., 1979). Similar application of ACh was ineffective. However, other studies have shown that microiontophoresis of ACh onto interneurons located in the dorsal horn produced both excitation and depression of these cells (Weight and Salmoiraghi, 1966; Myslinski and Randic, 1977).

The above findings indicate that NE, 5-HT, and CCh injected intrathecally could in theory effect thermoregulatory changes by acting within the dorsal horn. As mentioned previously, modulation of thermal afferent transmission through an effect on the dorsal horn should result in an apparent alteration in the thermoregulatory set-point. The findings in regard to the entire effect of 5-HT and the early portion of the CCh effect are commensurate with such a site and mode of action.

The apparent change in set-point associated with the 5-HT effect and the early part of the CCh response could also have been produced by modulation of input from spinal thermodetector units. There exists good evidence that the thoraco-lumbar cord contains thermodetector neurons similar to those located in the AH/PO (see Introduction), although the anatomical location within spinal tissues of these units remains to be determined. Thermal information from spinal thermosensitive structures is transmitted to

supraspinal sites by the spinothalamic tract (Wunnerberg and Bruck, 1970; Simon and Iriki, 1971). Thus, thermodetector units probably synapse with dorsal horn interneurons or directly with spinothalamic cells. 5-HT, and possibly CCh, might modulate the input from spinal thermodetectors by acting on these synapses. Alternatively, these substances might alter the firing rate of spinal detector units directly by interacting with appropriate receptors on the thermosensitive neuron.

Because intrathecally injected NE produced hypothermia and vasodilatation which were frequently temporally dissociated and because the hypothermia was not associated with inhibition of shivering-like activity, it was suggested earlier that this substance may have inhibited at the spinal level sympathetic outflow pathways controlling vasomotor tone and non-shivering thermogenesis. Preganglionic sympathetic neurons are located in the intermediolateral cell column of the spinal grey matter, and according to Ryall (1967), bulbospinal noradrenergic fibers terminating in the IML inhibit the activity of these neurons. Recent work by Coote and Macleod (1974, 1975, 1977) has supported the sympatho-inhibitory role for bulbospinal NE proposed by Ryall (1967). Thus, intrathecally injected NE might have caused hypothermia and vasodilatation by acting within the IML. In support of this suggestion, injection of clonidine

by several different central and peripheral routes has been shown to produce inhibition of sympathetic activity (Kobinger, 1978), and research indicates that the sympatho-inhibitory effect of clonidine is mediated by an action at spinal sites (Sinha et al., 1973; Smith, 1974; Dhawan et al., 1975). An inhibition of efferent sympathetic activity is consistent with the thermoregulatory effects of intrathecal NE and clonidine. In section B, results will be discussed from Phase II experiments which further support the contention that adrenergic agonists produce their effects on thermoregulation by decreasing sympathetic outflow from the IML.

Sympathetic outflow from the IML is segmentally distributed (Rubin and Purves, 1980; Rando et al., 1981); the cell groups controlling tail skin vasomotor tone and brown fat-mediated NST are located at different sites along the longitudinal axis of the cord. The two cell groups may not be equally accessible to intrathecally injected NE. A delay in the drug's reaching the portion of the IML controlling tail vasomotor tone may have been responsible for the tardy appearance of vasodilatation in many of the sessions.

The second phase of the CCh response was uncoordinated in the sense that shivering was present at the same time as tail skin vasodilatation. It is possible that the

vasodilatation was a consequence of an action on IML units. However, this speculation is not supported by microiontophoretic data. Application of ACh to preganglionic sympathetic neurons in the IML did not alter their firing rate (DeGroate and Ryall, 1967).

In regard to 5-HT, an action of this agent on the IML is unlikely as the thermoregulatory effects of 5-HT are more compatible with a dorsal horn site of action. Furthermore, intrathecal 5-HT produced effects which are consistent with an increase in sympathetic outflow, whereas a sympatho-inhibitory role for bulbospinal 5-HT has been suggested by Coote and Macleod (1974) and by Neumayr et al. (1974). However, if future research should indicate that 5-HT, in fact, acts on the efferent side of the thermoregulatory system, it would be necessary to explain how this agent can bring about an increase in shivering activity. One possibility is that 5-HT directly affects ventral horn mechanisms concerned with muscle tone. Recent miroiontophoretic studies have shown that 5-HT increases motoneuron excitability, and it has been suggested that bulbospinal serotonergic fibers which terminate in the ventral horn might regulate muscle tone (Parry and Roberts, 1980).

The shivering produced by intrathecal CCh might also be due to a direct action on the ventral horn. However, very little experimental evidence exists to support this

suggestion. In fact, Weight and Salmoiraghi (1966) have reported that ACh applied by microiontophoresis excites Renshaw cells. In light of the presumed inhibitory action of Renshaw stimulation on α-motoneuron-activity, one might expect that this type of effect would decrease shivering.

(v) The spinal cord as a potential site of action for NE, 5-HT, and CCh administered IVT or IC

Injection of NE (0.50-100 µg) IVT or IC produced hypothermia accompanied by tail skin vasodilation (Clark and Clark, 1980a), it has been assumed that the effects of NE injected IVT or IC are mediated by an action on hypothalamic structures. However, several investigators (Banerjee et al., 1970; Cantor and Satinoff, 1976) have suggested that this is not the case, and that the thermoregulatory effects of NE injected IVT and IC are produced by an action of this monoamine at extrahypothalamic sites.

Our data show that intrathecal injection of relatively low doses of l-NE (e.g., 5 µg) produced substantial hypothermia and a prolonged increase in T_{sk}. Moreover, previous research has shown that, following IVT and IC injections in rats and rabbits, significant amounts of radiolabeled NE reach the spinal subarachnoid space (Glowinski et al., 1965; Nowaczyk et al., 1978). Based on the above evidence, we suggest that the spinal cord is a

potential site of action which might augment or mediate the thermoregulatory effects of NE injected IVT or IC.

5-HT and CCh, following IVT and IC injection, probably also reach spinal tissues via the ventricular CSF circulation. 5-HT and CCh injected by either of the above routes most frequently produce hypothermia (Clark and Clark, 1980a, b). As our data show, intrathecal injections of these drugs induce hyperthermia. Consequently, subsequent stimulation of spinal serotonergic and cholinergic systems does not enhance or mediate the thermoregulatory responses to IVT or IC injections of 5-HT or CCh, respectively. However, the possibility exists that the effects of 5-HT and CCh injected into the supraspinal CSF are decreased or otherwise modified by an action at the spinal level.

(vi) Conclusions

The present study represents the first investigation of the effects on thermoregulation of aminergic and cholinergic agonists injected into the spinal subarachnoid space. Phase I experiments indicated that NE, clonidine, 5-HT, and CCh so injected produced clear-cut, dose-dependent changes in T_c and in the level of activity of certain thermoregulatory effectors. The data from Phase I also indicated that the thermoregulatory effects of 5-HT

and CCh injected intrathecally were due to a direct action of these agents on spinal sites, presumably the dorsal horn. However, the site of action for intrathecal NE was less well defined. It was suggested that the primary site of action for NE injected intrathecally was the IML but that the hypothermia mediated by the direct action of this monoamine on the cord was augmented by an ancillary action of NE at peripheral sites subsequent to leakage from the spinal subarachnoid space. In addition, it was suggested that the initial hyperthermia associated with the 0.30 µmole dose of NE was due to an action of this monoamine at peripheral sites mediating NST and vasomotor tone. These suggestions led to conception of the Phase II experiments, which were designed to ascertain the sites and mechanism of action for NE injected intrathecally.

The finding that intrathecal injections of NE, clonidine, 5-HT, and CCh produced changes in thermoregulation by an action at spinal sites provides pharmacological evidence that endogenous spinal cord monoaminergic and cholinergic systems might be involved in thermoregulation. However, further experiments are necessary to determine whether these spinal pathways play a physiologically relevant role in maintaining body temperature.

B. Phase II--the Sites and Mechanisms of Action of NE Injected Intrathecally

(i) Evidence that an action of NE at supraspinal sites is not responsible for the thermoregulatory effects of intrathecal injections of this monoamine

Studies of the distribution along the neuroaxis of intrathecally administered ^{3}H-NE indicated that, regardless of the time of sacrifice, the majority of the radioactivity recovered from the central nervous system (CNS) was localized to the spinal cord regions and that no more than 3% of the total injected activity was located in the brain. These results are similar to those of other studies examining the distribution of radiolabeled substances (i.e., morphine, ureas, naloxone) injected intrathecally (Yaksh and Rudy, 1976; Yeung and Rudy, 1980). Calculations based on the 0.30 µmole dose of NE (51 µg of base) injected intrathecally show that about 1.5 µg of NE base was found in the brain. Regarding the production of hypothermia, this amount of NE in the brain is less than the minimum effective dose of this monoamine given intracerebroventricularly (IVT) or intracisternally (IC) (Clark and Clark, 1980a). Considering the small amounts of NE which reached supraspinal sites, it is suggested that an action of NE in the brain was not responsible for the thermoregulatory changes induced by intrathecal administration of NE. Since

several agents with diverse pharmacokinetic characteristics did not reach the brain following intrathecal injection (this study; Yaksh and Rudy, 1980), it is likely that clonidine injected intrathecally also did not gain access to supraspinal tissues.

(ii) Evidence that an action of NE at peripheral sites mediates the hyperthermic response associated with the 0.30 µmole dose of intrathecal NE

Within three minutes after intrathecal injection of NE (0.30 µmoles), a relatively high level of unmetabolized NE (38.6 ng/ml) was detected in the plasma, indicating that as initial rapid escape of this monoamine from the subarachnoid space had occurred. The high plasma level was short lived; about 10-20 minutes after the first sample time, the plasma level of unmetabolized NE had decreased by 75%. The appearance of a high level of NE in the plasma correlated with the time when T_c was rising rapidly during the hyperthermia induced by the 0.30 µmoles dose, a finding which suggests that the hyperthermia may have been caused by peripheral actions of the circulating NE. Strong additional evidence in support of this notion is that, in rats pretreated with the ganglionic blocker mecamylamine, intrathecal administration of 0.30 µmoles NE induced a brief increase in T_c and a concomitant tail skin vasoconstriction.

The rise in T_c and fall in T_{sk} which occurred in the mecamylamine treated rats was presumably due to a direct action of NE at peripheral receptors mediating NST and tail skin vasoconstriction.

Several ancillary lines of evidence offer further support for the suggestion that, following leakage from the subarachnoid site of injection, NE acts at peripheral sites to produce hyperthermia: 1) Shivering was not observed during the hyperthermic response (Phase I experiments); 2) Intrathecal injection of NE did not increase the firing rate of the lumbar sympathetic chain; in fact, an initial, transient depression of firing rate occurred, which suggests a peripherally mediated reflex inhibition of the lumbar sympathetic chain (vide infra); and 3) The hyperthermia induced by i.v. infusion of NE in warm acclimated and cold acclimated rats (Depocas et al., 1978) occurred at plasma concentrations of unmetabolized NE which were less than those reported in this study.

(iii) Evidence that an action of NE at peripheral sites might contribute to the hypothermia induced by intrathecal injections of NE

In section A, it was suggested that the hypothermic phase of the response to intrathecal injection of NE might be partially mediated by an action of NE in the

periphery following escape from the site of injection. However, in that section several lines of evidence were cited which suggested that the hypothermia induced by intrathecal injection of NE, especially that caused by the lower doses, was produced mainly by an action of NE at spinal sites. In the first place, intrathecal injections of NE consistently produced hypothermia and a prolonged tail skin vasodilatation, whereas i.v. injections produced hypothermia in only 50% of the animals tested. Secondly, the tail temperature responses to i.v. NE was very brief compared to that produced by intrathecal NE. Finally, the hypothermia evoked by i.v. administration of NE was less than half as large as that elicited by the same doses given intrathecally. The Phase II experiments do not unequivocally answer the question of whether a systemic effect of NE contributes significantly to the hypothermia produced by NE injected intrathecally. However, it is noteworthy that the plasma NE concentrations during the hypothermia induced by the 0.03 and 0.06 μmole doses of NE were quite low (0.5-2 ng/ml), and even after the highest dose of NE, the amounts present in plasma during the hypothermia did not exceed 10 ng/ml. One might concede that it is possible that a plasma concentration of 10 ng/ml could lower core temperature, perhaps through a ganglionic blocking action (DeGroat and Volle, 1966), but it is very difficult to

believe that a concentration of 1 ng/ml could significantly affect thermoregulation. Furthermore, although the magnitude of the contribution of a peripheral action of NE remains to be determined, other results from the Phase II study suggest that a direct spinal action of intrathecally injected NE, i.e., an inhibition of sympathetic outflow possibly mediated through an action of the IML, contributed importantly to the hypothermic effect.

(iv) Effects of NE and clonidine on neural activity recorded from the lumbar sympathetic chain

The thermoregulatory effects of intrathecal NE and clonidine are consistent with an adrenergically induced inhibition of sympathetic outflow. The tail skin vasodilation produced by intrathecal NE and clonidine is a physiological effect which is clearly commensurate with a sympatho-inhibitory action of these drugs. The fall in T_c was probably mediated in part by heat loss associated with tail skin vasodilation. However, a decrease in NST resulting from an interruption of sympathetic outflow to intrascapular brown fat might also be involved. The data of Phase II show that intrathecal NE and clonidine produced prolonged depression of neural activity recorded from the lumbar sympathetic chain, whereas i.v. infusion of the same or larger doses of NE produced only transient inhibition.

These findings strengthen the hypothesis that NE- and clonidine-induced hypothermia and tail skin vasodilation were caused by a spinally mediated reduction in efferent sympathetic activity. On the other hand, it should be noted that the NE- and clonidine-induced hypothermia and tail skin vasodilation lasted longer than the depression of lumbar chain activity. The lack of a more exact temporal correlation could be a function of inertia inherent in the thermoregulatory system. Alternatively, the use of an anesthetic in the experiments in which lumbar chain activity was measured might have decreased the duration of drug effect in comparison to the effect on thermoregulation seen in unanesthetized animals (Caleresu et al., 1975).

The prolonged depression of lumbar firing rate produced by injection of NE was preceded by an initial transient decrease in lumbar activity (Fig. 11). This preliminary effect was probably a result of a baroreceptor-mediated reflex inhibition of sympathetic outflow consequent to leakage of NE from the spinal subarachnoid space into the systemic circulation. In support of this suggestion, the initial depression of firing rate is temporally correlated to the hyperthermia and the initial appearance of high concentrations of NE in the peripheral circulation following intrathecal injection (vide supra).

In the rat, the lumbar sympathetic chain is partially

postganglionic (Baum and Shropshire, 1973). Since NE has been found to inhibit ganglionic transmission (DeGroate and Volle, 1966), it might be suggested that the effects obtained with intrathecal injection of NE were mediated by ganglionic blockade subsequent to leakage of NE into the general circulation. However, the lack of temporal correlation between the prolonged depression of lumbar activity and the concentration of NE in the periphery does not support this suggestion; the plasma levels of NE were decreasing rapidly at the same time that the depression of lumbar firing rate was increasing.

Although only one dose of NE and clonidine was used in the present study, previous work (Phase I experiments) has demonstrated that NE (0.01-0.10 µmoles, N = 6) and clonidine (0.0175-0.070 µmoles, N = 4) injected intrathecally can induce dose-dependent, monophasic hypothermia associated with an increase in tail temperature. Since these dose-dependent changes in thermoregulation are consistent with an inhibition of sympathetic outflow, it is not unreasonable to suggest that intrathecal injections of NE and clonidine would cause dose-dependent inhibition of lumbar chain activity. However, further research is necessary to confirm this suggestion.

(v) Evidence that NE injected intrathecally produces its effects on thermoregulation by acting at the IML

It was suggested above and in section A, that the IML was the most likely spinal cord site which could mediate the thermoregulatory effects of NE and clonidine injected intrathecally. This suggestion was based on several considerations: 1) The changes in thermoregulation induced by intrathecal injection of the adrenergic agonists were consistent with an inhibition of sympathetic outflow; 2) Following intrathecal injection of NE and clonidine, T_c and T_{sk} were frequently out of phase, indicating that these changes were mediated by an effect on the efferent rather than the afferent side of the thermoregulatory system; 3) Research indicates that bulbospinal noradrenergic neurons terminating in the IML inhibit efferent sympathetic activity (DeGroate and Ryall, 1967; Coote and Macleod, 1974, 1975, 1977); and 4) Several investigators have suggested that the sympatho-inhibitory effects of clonidine are mediated by an action within the spinal cord (Sinha et al., 1973; Smith, 1974; Dhawan et al., 1975). In the Phase II experiment discussed above (see subsection iv above), it was shown that intrathecal injection of NE and clonidine elicited prolonged inhibition of efferent sympathetic activity. These data support the findings from previous

research and are consistent with the suggestion that the pharmacological effects of intrathecal NE and clonidine are due to an action of these drugs on the IML.

In the rat, the IML extends from T_1 to L_2 (Navaratnam and Lewis, 1970). The segmental organization of the preganglionic sympathetic fibers within this thoraco-lumbar region of the cord is well known, i.e., the cell groups controlling brown fat-mediated NST are located some distance rostrad to the cells controlling tail skin vasomotor tone. The studies with radiolabeled NE have shown that, in rats prepared with 8.5 cm spinal catheters, the majority of NE recovered from the CNS was associated with spinal tissue segments from the thoraco-lumbar region. Therefore, NE injected intrathecally in rats with 8.5 cm catheters had good access to regions of the IML controlling NST and peripheral vasomotor tone. If the IML is the spinal site at which intrathecal NE acts to produce its physiological effects, by altering the accessibility of NE to the IML, it should be possible to inhibit or at least modify the characteristic thermoregulatory changes.

In rats prepared with 4 cm and 12 cm spinal catheters, the T_c and T_{sk} responses to intrathecal NE (0.30 µmoles) were modified according to a definite pattern. In rats with 12 cm catheters, rather than being comparable to rats with 8.5 cm catheters, the hypothermia and accompanying

increase in T_{sk} were similar in both magnitude and duration to those elicited by i.v. NE (0.10 µmoles) (Figs. 14b and 14c) (i.e., NE injected i.v. and injected intrathecally in rats with 12 cm catheters caused small falls in T_c which were similar in duration and transient increases in T_{sk} of like magnitude). Moreover, the CNS distribution of NE following intrathecal injection in these rats was confined to the caudal aspects of the cord. Thus, in rats with 12 cm catheters, the distribution of NE to the IML was limited, and the thermoregulatory effects of intrathecal NE in these rats may have been mediated primarily by the peripheral actions of this monoamine. In rats with 4 cm catheters, the hypothermic response to intrathecal injection of NE (0.30 µmoles) did not differ significantly from that of rats with 8.5 cm catheters. However, the T_{sk} response in rats with 4 cm catheters was not comparable to that seen in rats with 8.5 cm catheters, and instead resembled the effect of i.v. NE (0.10 µmoles) on T_{sk} (Figs. 14a and 14b). The majority of NE injected intrathecally in rats with 4 cm catheters remained localized to the cervical and upper thoracic area of the cord. Based on these data, it is suggested that by acting at thoracic IML areas controlling NST, NE injected intrathecally in rats with 4 cm catheters produced hypothermias comparable to those seen in rats with 8.5 cm catheters. However, in the rats with 4 cm catheters,

the distribution of NE is such that this monoamine does not influence caudal areas of the IML which regulate sympathetic outflow to the tail. Consequently, only the transient change in T_{sk} associated with the peripheral effects of NE were observed. These results are consistent with the suggestion that the hypothermia and vasodilation induced by intrathecal injection of NE near the lumbar enlargement are mediated primarily by an action of this monoamine in the IML of the spinal grey matter.

Comparisons of the initial hyperthermic responses showed that the rise in T_c produced by intrathecal injections of NE (0.30 µmoles) in rats with 12 cm or 4 cm catheters did not differ statistically from that elicited in rats with 8.5 cm catheters. However, the hyperthermia induced by NE in rats with 12 cm catheters was significantly different from that associated with rats possessing 4 cm catheters. Assuming that the hyperthermia is due to an action of NE at peripheral sites, then this response should be similar regardless of catheter length. It could be suggested that the hyperthermia was mediated by an action of NE at spinal sites (possibly a nonspecific, high dose effect). In light of other evidence (vide supra), this possibility does not seem likely. These data may indicate that the potential for the efflux of NE into the periphery is not a consistent phenomenon along the

subarachnoid space.

(vi) Conclusions

The research of Phase II represents an attempt to ascertain the sites of thermoregulation of NE injected intrathecally at the level of the lumbar enlargement and to provide evidence for the mechanism by which NE so injected might produce changes in thermoregulation. The results from this phase indicate that the hypothermia produced by intrathecal injection of NE was mediated primarily by a direct action of this monoamine at the IML causing a reduction of sympathetic outflow. However, an action of NE in the periphery following leakage from the subarachnoid site of injection might augment the developing hypothermia and is probably totally responsible for the hyperthermia associated with the 0.30 µmole dose of intrathecal NE. These findings provide further pharmacologic evidence that bulbospinal noradrenergic pathways terminating in the IML play a pertinent role in thermoregulation.

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