ANALYSIS OF TEMPERATURE-DEPENDENT SENSITIVITY OF ISOLATED ATRIA TO ADRENERGIC AGONISTS AND ANTAGONISTS

BY

HECTOR MUNOZ-RAMIREZ

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INTRODUCTION

Temperature has a definite and universal influence in biological phenomena. In living organisms, the importance of temperature is illustrated by the relatively constant temperature found in the higher forms of life. The state of constant temperature (homeothermia) is maintained by the interplay of central and peripheral mechanisms since, as Dubois (1941) indicated, "temperature regulation is too vital to be entrusted to any one small center in the brain". In essence, the temperature of the tissues is the result of heat production and heat loss regardless of the mechanisms involved in the production and dissipation of heat.

Temperature has played an important role in evolution, especially in the early states of life. Burton and Edholm (1955) described the role of temperature in the following manner: "We are told that the world was at one time populated by poikilothermic animals of fantastic size. Yet their life must have been at the mercy of the climate. On a cold day the battle of existence slowed to relative inactivity. . . Perhaps the dependence of the large poikilotherms upon the temperature was the chief factor in the extinction of most of their species, once they had to compete with others less powerful but enjoying thermodynamic freedom".

The influence of temperature on chemical reactions

All chemical reactions change their rate with a change

of temperature. Arrhenius (1907) determined the quantitative effect of temperature on the rate of hydrolysis of sucrose in the presence of various acids. He observed that the rate of hydrolysis of sucrose could not be explained in terms of the effect of temperature upon the kinetic energy of the molecules. He then derived the relationship between temperature and rate. From this equation it can be shown that there is a linear relationship when the natural logarithm of the rate is plotted against the reciprocal of the absolute temperature. Since life depends on a complex network of chemical reactions, it follows that an effect of temperature on living organisms may be exerted by altering chemical reaction rates. In living organisms these chemical reactions are usually brought about by specific enzymes. Some of the ways by which temperature can affect the velocity of enzyme reactions are: a) by affecting the stability of the enzyme, b) by affecting the actual breakdown of the enzyme-substrate complex, c) by affecting the enzyme-substrate affinity, d) by affecting the pH functions of any or all of the components of a reaction, or e) by affecting the affinity of the enzymes for activators or inhibitors (Dixon and Webb, 1960).

When enzymes are subjected to high temperatures, activity is usually lost. However, the activity may be recovered if the temperature is not too high and the time of exposure not too long. For instance, in 1913 Mellanby and Wooley showed that acid solutions of trypsin (pancreatic juice) could be heated to boiling temperatures for approximately 5 minutes

followed by subsequent recovery of activity at ambient temperature. Similar findings were reported by Northrop (1932) using crystalline trypsin.

The significance of temperature denaturation of proteins as a factor influencing biological processes was studied in luminous bacteria. When the brightness of bacterial luminescence was plotted versus temperature, the intensity of luminescence increased with temperature until a maximum was reached and then diminished rapidly (Brown et al., 1942). It was also observed that the optimum temperature was different for different species of bacteria and that the decline in intensity above optimum was reversible on cooling. Luminescence is the manifestation of the reaction between substrate luciferin and enzyme luciferase.

By this time the Arrhenius rate theory had already evolved on strong grounds. Arrhenius himself (1907) extended his formulation to the rate of biochemical reactions by calculating activation energies for spontaneous destruction of pepsin and trypsin solutions. In 1942 Eyring and Magee, using the data of Brown and his associates, applied the rate theory to bacterial luminescence. In a simple definition, the rate theory states that every rate process, whatever its nature, is in fact an unstable equilibrium between an activated complex and the reactants (Eyring and Magee, 1942). They concluded that the relationship between bacterial luminescence and temperature could be interpreted quantitatively according to the Arrhenius equation.

The observation that the decreased intensity of luminescence was entirely reversible on cooling, provided the high temperature (in these experiments 40° C) is not maintained too long, led to the conclusion that neither thermal destruction of the enzyme nor a killing of the cells was responsible for the decrease in bacterial luminescence (Brown et al., 1942).

General effects of low temperature on mammals

The capacity of animals to support low ambient temperatures varies among species. Animals were exposed to cooled air at a temperature of -35° C. Under these conditions, mice survived 0.4 hours, rats 0.75 to 2 hours, rabbits 3.5 to 6.5 hours and pigeons 22 to 78 hours (Horvath et al., 1948). It should be pointed out that in this type of experiments the temperature of the animal is not the same as the environmental temperature.

Studies of the effect of temperature on mammals have been carried out mainly on animals maintained with low body temperatures. These studies were performed in light of uses of low temperatures, e.g. performance of heart surgery in a bloodless field (Bigelow, 1950), cancer therapy (Fay et al., 1938; Smith, 1942), and treatment of schizophrenia (Smith, 1942). Another reason for studying the effects of hypothermia was the need to determine tolerance and to evaluate methods of survival in humans exposed to considerable low temperatures (Molnar, 1946).

Animals can experience a considerable fall in body temperature and survive. Rodents have been cooled to body temperatures of approximately 0° C and subsequently survived (Andjus and Smith, 1955). Gollan, in 1954, successfully revived dogs maintained at 0° C even after cardiac arrest had occurred at the low temperature. In these animals blood circulation was artificially maintained. With respect to the effects of low temperature in the cardiovascular system, Arkell and Waughan (1940) observed, in humans, that both heart rate and blood pressure decreased at a rectal temperature of 32° C. However, an initial rise in heart rate and blood pressure occurred at the beginning of hypothermia (Talbot et al., 1941). In rats there is also an initial increase followed by a decrease in these cardiovascular parameters as the rectal temperature is decreased to 30° C and 23° C (Adolph, 1950). The response pattern in regard to heart rate and blood pressure has been recently confirmed in dogs by Halkola et al. (1972). They also observed that propranolol blocked the early positive chronotropic effect induced by hypothermia. This indicates that the increase in heart rate could be the result of increased discharge of endogenous catecholamines. In fact, urinary excretion of catecholamines has been shown to increase in rats exposed to low environmental temperatures (Leduc, 1961; Leblanc and Vadeau, 1961).

The hypothermia-induced decrease in heart rate appears to be due to a decreased temperature of the atrial pacemaker.

In cooled rats, Crismon and Elliot (1947) observed that warming the heart by an electric heater located in the esophagus produced an increase in heart rate and blood pressure.

Some discrepancy exists with regard to oxygen consumption during hypothermia. Species differences and different experimental designs can be cited as reasons for such discrepancy. Zakhary et al. (1967) protected newborn dogs from severe induced asphyxia by reducing the body temperature to 15^{0} C. They indicated that the protection achieved was due to lower oxygen requirements during hypothermia. However, in humans, Adamson et al. (1965) found that oxygen consumption increased when the temperature of newborn infants was reduced to 33^{0} C. The increased oxygen consumption appears to be associated with the shivering that accompanies hypothermia. Experiments by Koivikko and Länsimies (1972) show that in anesthetized young lambs and dogs, oxygen consumption diminishes as temperature decreases. In the absence of anesthesia their data show an initial increase in oxygen consumption followed by a progressive decrease. However, the rate of decline of oxygen consumption was smaller than in the anesthetized animals.

Shivering appears to be related to glucose plasma levels. In hypothermic dogs the intravenous administration of glucose induced a shivering state (Cassidy et al., 1925). Insulin blocked the shivering state produced by glucose.

Changes in CO_2 blood levels are found during hypothermia. Cranston et al. (1955) observed a definite increase in blood

CO_2 levels when the rectal temperature was decreased to 25° C. At the same time, they recorded an increase in blood hydrogen ion concentration. It has been pointed out that because of the shift to the left of the hemoglobin dissociation curve observed at low temperatures, oxygen would be less available for release to the tissues (Werz, 1943). However, the shift to the right of the hemoglobin dissociation curve produced by acidosis would tend to counteract this effect of low temperature (Comroe et al., 1959). In anesthetized hypothermic dogs, Hegnauer and D'Amato (1954) found that, at a rectal temperature of 17° C, the heart cells were receiving adequate oxygenation.

Drastic electrolyte changes may also occur when the temperature of an animal is decreased. A change in ionic ratio may be manifested in physiological alterations of the tissues. The shifts in electrolytes produced by low temperature could be secondary to hepatic phenomena. For example, increased levels of catecholamine at low temperatures (Leduc, 1961; Leblanc and Vadeau, 1961) could lead to increased release of potassium from the liver (Craig and Honig, 1963). Also, low temperature may influence the energy generating processes, thereby affecting energy dependent ionic gradients. In dogs plasma levels of sodium were not significantly altered when the rectal temperature was decreased to 20° C (Swan et al., 1953; Fleming, 1954). In contrast, plasma potassium levels in dogs were consistently increased when the body temperature was decreased to 20° C (Bigelow et al., 1950). At a rectal

temperature of 25° C there was also an increase in plasma potassium levels in rats (Elliot and Crismon, 1947). The increase in plasma levels of potassium is not, however, a general finding. Fleming (1954) and Deterling et al. (1955) found no difference in the plasma potassium levels between normothermic and hypothermic dogs. It is of interest that Swan et al. (1953) reported a development of hypokalemia when dogs were hyperventilated at normal temperatures. They found, however, no correlation between concentration of CO_2 and plasma potassium levels.

Elliot and Crismon (1947) found an increase in serum calcium in the rat when the rectal temperature was reduced to 25° C. An increase in plasma levels of calcium was also reported in dogs at a rectal temperature of 20° C (Bigelow et al., 1950). However, Fleming (1954), studying the same species, found no significant change in plasma concentration of calcium when the rectal temperature was lowered to 20° C.

Effect of temperature on tissue electrolytes

It is unlikely that determination of plasma levels of different electrolytes provides true insight into ionic changes that can occur at the tissue and cellular level upon decreasing temperature. The use of isolated tissues would be more advantageous to follow temperature-dependent ionic changes because the extracellular concentration of an element can be controlled. When most tissues are cooled, they undergo a loss of cellular potassium and a simultaneous gain in

sodium concentrations. For example, Calkins et al. (1954) found that incubation of rat diaphragms in Krebs-Ringer solution at 2° C lowered potassium content from 90 mM/g to 40 mM/g. They speculated that glycolytic processes, required to maintain the high levels of intracellular potassium, were altered by low temperatures. Qualitatively similar results were found in human erythrocytes when incubated at 5° C. More recently, Mendler et al. (1971) perfused rat hearts at 4° C for four hours and found that tissue sodium increased by 300% while tissue potassium decreased by 70%. They also found signs of membrane deterioration such as cell swelling and a positive correlation between water uptake and potassium loss. In guinea-pig taenia coli, tissue potassium content decreased from 75 μmole/g to 17 μmole/g at a bath temperature of approximately 13° C while sodium content was inversely altered (Raisin and Gulati, 1972). The isolated dog heart perfused with oxygenated blood at 13° C showed a decrease in potassium content from 34 to 25 mEq/100 g dry weight, whereas the sodium content increased from 16 to 30 mEq/100 g dry weight (Bui-Mong-Hung et al., 1972). In this study there was no impairment of high energy phosphate stores, but drastic histological changes like cell swelling and membrane distortion were observed. Using radiotracers to determine the effect of temperature on electrolyte fluxes, Langer and Brady (1968) found that cooling rabbit ventricular muscle below 20° C produced a decrease in intracellular potassium and an increase in intracellular sodium and calcium. They suggested

that the major factor responsible for the electrolyte changes was a decreased activity of the sodium pump at low temperatures. In rat uterus there was a net gain of sodium after 60 minute incubation period at 5° C in Krebs-Ringer solution (Daniel and Robison, 1971). It was suggested that, at low temperature, sodium is bound to ionic sites originally occupied by potassium. The release of potassium from these cytoplasmic sites may result from ATP depletion produced at low temperature. However, when iodoacetate was used, to deplete ATP stores, there was less release of potassium than produced by low temperature.

The changes in tissue content of electrolytes observed at low temperatures are influenced by the experimental conditions and by the degree of cooling. Storage of rat heart at 4° C for four hours produced less intense electrolyte changes than when hearts were perfused at the same temperature (Mendler et al., 1971). The most prominent electrolyte changes are observed at temperatures between 15° C and 1° C. Experiments by Reisin and Gulati (1972) show that at 20° C the potassium content of the guinea-pig tenia coli is 9 μmole/g less than at 37° C whereas at 13° C it is 58 μmole/g less. At 25° C there was no significant gain in sodium content of rat uteri compared to 37° C (Daniel and Robison, 1971). Similarly, it was not possible to define any electrolyte change in rabbit ventricular muscle at a temperature range between 20° C and 37° C (Langer and Brady, 1968).

It should be pointed out that cooling of the tissue is

not the only method that leads to electrolyte changes. An increase in the frequency of contraction of dog papillary muscle, at normal temperatures led to a loss of intracellular potassium and a gain in intracellular sodium and calcium (Langer, 1967). Mechanical manipulation of the aorta produced a loss of two-thirds of the tissue potassium and an increase in sodium from 275 to 353 mEq/kg during the first 2 minutes after removal of the tissue (Dawkins and Bohr, 1960). These investigators suggested that handling the tissue may result in alteration of cell barriers leading to electrolyte changes.

Temperature-dependent sensitivity (TDS) to catecholamines

Langlois (1898) first reported that alteration of body temperature changed the sensitivity of cardiovascular smooth muscle to catecholamines. He observed that decreasing the body temperature of cats from 38° C to 35° C resulted in prolongation of the pressor response to suprarenal extracts. Local cooling of the eye of the anesthetized cat resulted in decreased rate of relaxation of the nictitating membrane previously contracted by stimulation of the postganglionic cervical sympathetic nerve (Elliot, 1905). Elliot also observed that cooling the whole animal to 31° C abolished the contracture of the nictitating membrane produced by postganglionic nerve stimulation although somatic motor nerve function was still intact. Therefore, low temperature apparently selectively depresses conduction along sympathetic neurons

(Elliot, 1905). However, Khan et al. (1965) found that the response of the isolated guinea-pig atrium to stimulation of the parasympathetic nerve supply was decreased or abolished by decreasing the temperature to a point where responses could still be elicited by stimulation of the sympathetic innervation. Furhman et al. (1944) examined the effect of intraarterially or intravenously injected epinephrine and found that the duration of the response of the nictitating membrane became progressively longer as the body temperature was reduced from 43° C to 13° C. This prolongation of response was abolished by local warming of the liver (Furhman et al., 1944). These results suggested that enzymatic inactivation of epinephrine is decreased by low temperature.

The experiments of Elliot attracted the attention of many investigators motivated by the potential uses of hypothermia. For example, in Raynaud's disease, a condition characterized by constriction of blood vessels of feet and hands, one of the interesting features is that patients show extreme susceptibility to cold. The mere handling of a cold object can precipitate an attack resulting in the loss of fingers from gangrene produced by prolonged vasoconstriction (Ascroft, 1937). In years prior to 1940, treatment of patients with Raynaud's syndrome consisted in destruction of the sympathetic innervation to the feet and hands. In an experimental analysis of the effects of this treatment for Raynaud's disease, it was found that after denervation of one

of the upper limbs in the monkey, reduction of ambient temperature produced constriction of blood vessels in both the normal and denervated limb. However, when the ambient temperature was increased, dilation of the sympathectomized vessels was delayed and took place more slowly than on the normal side (Ascroft, 1937). It was also observed that vasoconstriction induced by combined epinephrine and cold was greater than the vasoconstriction induced by either treatment alone.

It is surprising that sympathectomy was the treatment advocated for Raynaud's disease. When the sympathetic nerves to an organ are severed, increased sensitivity to catecholamines occurs (Trendelenburg, 1963). Removal of sympathetic innervation may result in further development of sensitivity (Cannon, 1937). Lewis and Landis (1929) observed arterial vasoconstriction after exposure to cold in patients with Raynaud's disease even after sympathectomy. They concluded that constriction of digital vessels produced in Raynaud's disease by local cold is not due to abnormal vasomotor impulses.

Increased sensitivity of denervated blood vessels to cold was also demonstrated by Perkins et al. (1947). Using the skin temperature as an index of vascular diameter, they observed that, in the denervated hind paw of the cat, vasoconstriction occurred between 19° C and 26° C when examined within 2 days after the operation. By 21 days after denervation, vasoconstriction occurred between 28° C and 31° C.

In other words, the smooth muscle is more sensitive to cold at 21 days postoperatively. Similar results in adrenalectomized animals (Perkins et al., 1948) indicate that circulating catecholamines do not play a major role in this phenomenon.

Even though one may question the completeness of denervation in the above mentioned experiments, the increased sensitivity to cold, manifested by persistent vasoconstriction in certain pathological conditions, is unlikely to be associated with increased sympathetic discharge. It should be emphasized that very low temperatures produce a decrease in neuronal conduction. Neuronal conductivity in the cat spinal cord is abolished at a body temperature of 20° C and diminished between 37° C and 20° C (Brooks et al., 1955). It is possible, then, that the increased sensitivity of smooth muscle to catecholamines at low temperatures may be due to slower disappearance of amines and/or alteration of the muscle fiber as suggested by Elliot (1905).

The increased sensitivity of cardiovascular smooth muscle to cold observed in adrenalectomized animals led Perkins et al. (1948) to suggest that other substances might be involved in this phenomenon. In recent studies, Vanhoutte and Shepherd (1970) found that the contraction induced by serotonin, acetylcholine, and adenosine triphosphate in isolated cutaneous veins of dogs was potentiated by cooling. These data suggest that TDS is not limited to catecholamines. This type of supersensitivity is similar to that observed in

the decentralized and denervated nictitating membrane in the sense that both are relatively unspecific (Langer et al., 1967).

Although temperature definitely influences the response to drugs, the whole animal interpretation of the mechanisms involved is difficult because of factors such as absorption, distribution, neuronal and humoral reflexes which may all be altered at low temperatures. Moreover, results from toxic effects of drugs at low temperature do not provide information regarding basic phenomena. Another situation which may lead to confusion is when experiments are carried out at low environmental temperatures which do not change body temperature. For example, when the environmental temperature was increased from 23° C to 35° C the LD_{50} of epinephrine in mice decreased 14 times, that of norepinephrine 2.3 times, but there was no change in the toxicity of isoproterenol (Richards et al., 1970).

Ventricular fibrillation is a major risk at low body temperatures. Numerous factors have been implicated in the production of cardiac fibrillation at low temperatures. There are several reasons to believe that adrenergic mechanisms play an important role. Furhman et al. (1944) demonstrated that during hypothermia, ventricular fibrillation in cats was produced by doses of epinephrine that produced no cardiac irregularities at higher temperatures. In vivo studies with cats have shown that elimination of endogenous catecholamines, either by administration of reserpine or by

cardiac sympathectomy, prevented ventricular fibrillation during hypothermia (Nielsen and Owman, 1968; 1969). In isolated cat hearts, catecholamines consistently induced ventricular fibrillation when perfused at a temperature of 21° C. The same doses produced only positive inotropic and chronotropic responses at 37° C (Falk et al., 1972). It has been suggested that cooling produces activation of sympathetic neurons and release of catecholamines which, combined with increased sensitivity of the myocardium, leads to ventricular fibrillation. Locally released catecholamines appear to be more important than circulating catecholamines in this phenomenon, since cardiac sympathectomy prevents the ventricular fibrillation (Nielsen and Owman, 1969).

Various studies have demonstrated that low temperature increases the sensitivity of cardiac tissue to the chronotropic and inotropic effects of catecholamines. Thus, Garb and Penna (1956) pointed out that cooling isolated hearts not only decreased oxygen consumption and glucose metabolism, but, in the isolated cat and guinea-pig atria, enhanced the sensitivity to the positive chronotropic and inotropic effects of isoproterenol. Schneider and Gillis (1966) confirmed this finding and also demonstrated that the sensitivity to the positive chronotropic effect of norepinephrine and sympathetic nerve stimulation in isolated cat atria was increased by decreasing the bath temperature to 30° C. Oppermann et al. (1971) reported that isolated guinea-pig atria were 4 times as sensitive to the chronotropic effect of

norepinephrine at 26° C than at 37° C. This confirmed previous observations of Trendelenburg (1968). In contrast, Broadley (1972) was unable to detect any change in sensitivity to the chronotropic effect of catecholamines in the isolated perfused guinea-pig heart at temperatures between 38° C and 20° C. The effect of temperature on the tissue sensitivity to catecholamines appears to be species dependent. In isolated perfused rabbit heart the sensitivity to the chronotropic effect of epinephrine was greater at 37° C than at 25° C or 18° C (Fuhrman, 1946; Booker, 1960). Subsensitivity to the chronotropic effect of norepinephrine was also observed in isolated spontaneously beating rabbit right atria at 26° C (Oppermann, 1970). No explanation for this subsensitivity has been determined.

Possible mechanisms of the temperature-dependent sensitivity (TDS)

Several mechanisms may be involved in the increased sensitivity of isolated tissues to catecholamines at low temperatures. These mechanisms are: 1) alteration of adrenergic neuronal or extraneuronal uptake, 2) alteration of activities of mono amine oxidase (MAO) or catechol-O-methyl transferase (COMT), 3) changes in conformation of receptors, 4) alteration of some step in the stimulus-response coupling mechanism (Broadley, 1972; Oppermann et al., 1972; Reinhardt et al., 1972).

Uptake into adrenergic nerve terminals is the major

mechanism for terminating the action of norepinephrine. Neuronal uptake, also known as uptake$_1$, is stereoselective in some tissues and shows saturation kinetics (Iversen, 1967). A different type of uptake process, the extraneuronal uptake or uptake$_2$, was described by Iversen (1965) in isolated perfused rat hearts. The two processes have different substrate structural requirements. For example, while extraneuronal uptake has high affinity for isoproterenol, neuronal uptake has low affinity for this amine. In isolated rabbit ear artery Gillespie and Towart (1972) demonstrated that extraneuronal uptake also obeys saturation kinetics. In contrast, in the isolated nictitating membrane Trendelenburg *et al*. (1971) failed to demonstrate saturation of extraneuronal uptake.

Reduced activity of neuronal uptake at low temperature would allow accumulation of norepinephrine in the so called biophase or space immediately adjacent to the receptors. This, in turn, is considered to lead to an increase in observed potency of the adrenergic amine. It has been demonstrated that neuronal uptake of norepinephrine diminishes with decreases in temperature (Molinoff and Axelrod, 1971). Moreover, in guinea-pig atria, neuronal accumulation of norepinephrine was reduced at 26° C (Oppermann *et al*., 1971). Similarly, Sachs (1970) demonstrated that in mouse atria neuronal accumulation of norepinephrine was reduced by approximately 50% at 26° C as compared to 37° C. Thus, the dependence of neuronal uptake upon temperature seems to be a

common feature for different mammals. However, in isolated ventricle strips of frogs (rana pipiens) accumulation of norepinephrine in adrenergic nerve terminals seems to be the same at 15° C, 25° C and 35° C (Martinez-Sierra and Lorenzo-Velazquez, 1972).

A reduction of neuronal uptake of norepinephrine, produced by low temperature, could explain the increased sensitivity of isolated tissues to norepinephrine. However, when neuronal uptake was blocked by cocaine, TDS was still observed (Trendelenburg, 1968; Oppermann et al., 1971). It appears, then, that alteration of the neuronal uptake may not be the only factor involved in TDS.

Supersensitivity to agonists not taken up by adrenergic nerve terminals has also been observed at low temperatures. Isoproterenol is an example of such an agonist (Iversen, 1967). Isoproterenol dose-response curves are shifted to the left in a variety of tissues when the bath temperature is decreased (Garb and Penna, 1956; Schneider and Gillis, 1966; Oppermann et al., 1971; Reinhardt et al., 1972; Broadley, 1972; Wagner et al., 1972a; Schümann et al., 1972). It is clear that alteration of the neuronal uptake mechanism would not be expected to play a significant role in the development of TDS to this agonist.

It is now accepted that isoproterenol is taken up by an extraneuronal mechanism which is blocked by phenoxybenzamine and 3-O-methylated derivatives of norepinephrine, epinephrine and isoproterenol (Iversen, 1967; Salt, 1972). Recently,

Gillespie et al. (1970) demonstrated that the extraneuronal uptake of norepinephrine in cat spleen was decreased by low temperatures. Then it follows that TDS to isoproterenol could be explained on the basis of a reduction of extraneuronal uptake at low temperature. However, Wöppel (1972, quoted by Reinhardt et al., 1972) observed that phenoxybenzamine did not influence the TDS to beta sympathomimetic amines suggesting that extraneuronal uptake may not be involved in the phenomenon.

The importance of extraneuronal uptake in terminating the action of catecholamines is not fully established. In rat heart, high concentrations of norepinephrine were required to demonstrate extraneuronal uptake (Iversen, 1965). Similarly, in mouse atria, extraneuronal uptake was observed only at high concentrations of norepinephrine (Sachs, 1970). Recently, it has been shown that extraneuronal uptake also functions at low concentrations of catecholamines (Lightman and Iversen, 1969). Inhibition of COMT resulted in the lowering of the threshold for extraneuronal uptake of norepinephrine in rat heart (Lightman and Iversen, 1969; Einsenfeld et al., 1967a), rabbit ear artery (Burnstock et al., 1971), and cat nictitating membrane (Draskoczy and Trendelenburg, 1970; Trendelenburg et al., 1971). The increased extraneuronal uptake of norepinephrine after COMT inhibition was explained by Lightman and Iversen (1969) as an indication that, under control conditions, low concentrations of the adrenergic amine were so rapidly degraded that detection of

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was still present although to a lesser extent as compared to normal tissue (Jarrot, 1971a). This was taken as an indication that MAO is localized both intra- and extraneuronally in some tissues. The ratio of neuronal to extraneuronal MAO activity varies among tissues. In the cat nictitating membrane, chronic denervation produced a 70% fall in the total MAO activity (Burn and Robinson, 1953). In contrast, chronic denervation produced only in MAO activity in dog heart (Potter et al., 1963), cat iris and foreleg (Burn et al., 1954). In immunosympathectomized mice, a decrease in MAO activity was found only in the spleen (Jarrot, 1971a). In other tissues of the mouse such as heart, kidney, and small intestine, there was no significant alteration in the enzymic activity. In this study Jarrot could not detect any norepinephrine in the hearts of the animals, indicating that immunosympathectomy was essentially complete. Jacobowitz (1972) found a significant proportion of MAO activity of guinea-pig heart associated with ventricular fibroblasts.

Jarrot (1971a) suggested that the decrease of MAO activity in different tissues was closely associated with sympathetic innervation e.g., the denser the sympathetic innervation of an organ the larger the fall in the enzyme activity after denervation. MAO activity and density of adrenergic innervation appear to be directly related in chick amnion and human umbilical artery, although no consistent pattern was found in other tissues (Burnstock et al., 1972). High activity of MAO is not necessarily an indication of the

density of adrenergic innervation. In guinea-pig gastrointestinal tract (stomach, small intestine, and proximal colon) high enzyme activity is associated with ganglion cells of non-adrenergic nerves in the myenteric plexus (Furness and Costa, 1972).

Burn and Robison (1953) postulated that supersensitivity could result when MAO activity is decreased. They found that after chronic denervation of the cat nictitating membrane MAO activity was reduced and the sensitivity of this tissue to catecholamines was increased. Rebhun et al. (1954) found that iproniazid enhanced catecholamine-induced neurological responses (tremors, salivation, lacrimation, urination, opisthotonus) in guinea-pigs. MAO inhibition did not potentiate responses to agonists which were not substrates for MAO. In vitro studies have also demonstrated increased sensitivity to catecholamines after MAO inhibition. The MAO inhibitor pheniprazine sensitized isolated left atrium from reserpine pretreated guinea-pigs to the inotropic effect of norepinephrine (Furchgott and Sanchez-Garcia, 1968). In contrast, MAO inhibition by pargyline failed to increase the sensitivity of isolated guinea-pig atria to the chronotropic effect of norepinephrine (Trendelenburg, 1968). Similarly, Antonaccio and Smith (1969) were unable to detect sensitization to the chronotropic and inotropic effects of norepinephrine in isolated atria from pargyline pretreated guinea-pigs. Using the oil immersion technique, Kalsner and Nickerson (1969) demonstrated that inhibition of MAO by iproniazid did

not alter the relaxation of rabbit aortic strips in oil. This was interpreted as an indication that MAO contributes little to the inactivation of catecholamines. Therefore, the supersensitivity to catecholamines observed after MAO inhibition appears to depend on the experimental conditions and the tissue studied.

Reduced activity of MAO at low temperatures could account for the TDS to catecholamines. In vitro measurements of MAO activity of cat atria showed a 19% decrease in product formation at 30% (Schneider and Gillis, 1966). In guinea-pig atria the MAO activity was reduced by 28% at 26° C (Oppermann et al., 1972). The latter investigators considered the possibility that this reduction in enzymatic activity could explain the TDS, provided that the effect was sufficient to produce increased levels of norepinephrine at the receptor. They found however, that prior inhibition of MAO by pargyline had no influence on the TDS.

In 1957, Axelrod observed that norepinephrine and epinephrine were metabolized to the corresponding 3-O-methylamines. Axelrod and Tomchick (1958) reported the presence of the enzyme catechol-O-methyltransferase in rat liver. They demonstrated also that COMT requires S-adenosylmethionine as a methyl donor and a divalent cation such as Mg^{2+}.

COMT is found primarily in the soluble fraction of tissue homogenates, although small amounts are also present in the microsomal fraction (Inscoe et al., 1965) and in fat cell membranes (Traiger and Calvert, 1969). The enzyme appears

to be present mainly outside the sympathetic neuron in some tissues, since immunosympathectomy did not alter the COMT activity in rat and mouse heart (Iversen et al., 1966). Furthermore, O-methylated metabolites of norepinephrine increased in hearts from immunosympathectomized rats (Iversen et al., 1966) and in the denervated nictitating membrane (Draskoczy and Trendelenburg, 1970). In guinea-pig ventricles Jacobowitz (1972) found the fibroblasts as one of the extraneuronal sites of COMT activity. However, recent studies of the norepinephrine metabolizing enzymes showed that a significant proportion of COMT activity of rat and rabbit vas deferens, but not of heart and spleen, appeared to be associated with the sympathetic nerves (Jarrot and Iversen, 1971; Jarrot, 1971b). These investigators found a fall in COMT activity after denervation of rat and rabbit vas deferens.

Bacq et al. (1959) reported that inhibition of COMT by pyrogallol or catechol increased the sensitivity of several smooth muscles of the cat to epinephrine. In isolated rabbit heart, pyrogallol increased the sensitivity to the positive chronotropic and inotropic effects of norepinephrine (Juorio et al., 1963). In contrast, the sensitivity to the chronotropic and inotropic effects of norepinephrine was unaltered after inhibition of COMT by U-0521 (Giles and Miller, 1967). Foster (1967) also failed to observe an increase in the sensitivity of guinea-pig trachea to norepinephrine after inhibition of COMT by pyrogallol. On the other hand, in experiments with cat isolated papillary muscle, an increase in

sensitivity to norepinephrine after block of COMT by U-0521 was observed if the tissue was exposed to cocaine (Kaumann, 1970). In rabbit heart, U-0521 increased the sensitivity to the inotropic and chronotropic effects of isoproterenol (Giles and Miller, 1967). Isoproterenol, unlike norepinephrine, is not taken up by adrenergic nerve terminals (Iversen, 1967).

Therefore, it appears that metabolism by COMT is an important factor in determining the sensitivity to catecholamines when neuronal uptake is not present. In tissues such as the rabbit aorta which have sparse adrenergic innervation (Bevan and Verity, 1967), a small potentiating effect of cocaine has been observed (Levin and Furchgott, 1970). In this preparation metabolic inactivation by COMT has been postulated as the major factor terminating the action of catecholamines (Levin and Furchgott, 1970; Kalsner and Nickerson, 1969). However, the COMT activity of this tissue was scarcely detectable (Burnstock et al., 1972).

In isolated perfused rat heart, phenoxybenzamine and DCI blocked the O-methylation of catecholamines. When the tissue was homogenized these agents no longer inhibited O-methylation (Eisenfeld et al., 1967) suggesting that they may act by blocking the access of amine to the enzyme in intact tissue.

Increased sensitivity of isolated atria to catecholamines at low temperatures is believed to be due to decreased activity of COMT (Oppermann et al., 1971; 1972). However, in cat atria Schneider and Gillis (1966) found that COMT

activity was decreased only 17% when the bath temperature was lowered to 30° C. In guinea-pig atria, an 11° C decrease in bath temperature resulted in a 61% decrease in COMT activity (Oppermann et al., 1972). However, these latter investigators were unable to detect any change in the TDS to the chronotropic effect of norepinephrine after COMT inhibition by 3,4-dimethoxy-5-hydroxybenzoic acid (DMBA).

The hypothesis that decreased activity of COMT at low temperature is the factor responsible for TDS is in conflict with recent studies by Reinhardt et al. (1972). They demonstrated that the sensitivity of isolated guinea-pig atria to the inotropic effect of orciprenaline and Th 1165a, non-COMT substrates (Schmidt, 1971), was increased when the temperature was decreased from 42° C to 25° C. They postulated that TDS was due to a decrease in the metabolic rate of the tissue and not simply to diminished activity of COMT. Support for their conclusion came from studies using iodoacetate to block tissue metabolism. Under these conditions TDS was abolished (Schümann et al., 1972; Wagner et al., 1972b). The involvement of COMT in TDS was also questioned by Vanhoutte and Shepherd (1969) who found that isoproterenol-induced relaxation of vascular smooth muscle was enhanced by hypothermia even after inhibition of COMT.

TDS could also be explained by changes at the receptor level. Ahlquist (1948) first proposed to designate receptors as alpha and beta depending on the relative potencies of different catecholamines. For alpha receptors the order of

potencies is epinephrine > norepinephrine > isoproterenol. On the other hand for the beta receptors the order of potencies is isoproterenol > epinephrine > norepinephrine (Ahlquist, 1966).

The presence of alpha adrenergic receptors in heart tissue is controversial. Cotten and Walton (1951) suggested the presence of alpha adrenergic receptors in dog heart to explain the blockade produced by dibenamine of the inotropic effect of epinephrine. More recently, experiments by Govier (1967) demonstrated that low doses of phenylephrine, a predominantly alpha receptor agonist, produces myocardial contractions which are blocked by alpha receptor antagonists and unaltered by beta receptor antagonists. In contrast, Nickerson and Chan (1961) suggested that the blockade of the inotropic effect of epinephrine in cat atria by phenoxybenzamine was non-specific in nature, since this antagonist also blocked the inotropic response to calcium, a non-adrenergic receptor stimulant. The theory that both alpha and beta adrenergic receptors in heart tissue mediate the inotropic response is favored by experiments by Parr and Urquilla (1972). They observed that both alpha and beta receptor antagonists shifted the dose-response curves of adrenergic amines to the right. The involvement of alpha adrenergic receptors in the chronotropic action of epinephrine has been excluded. The positive chronotropic effect of phenylephrine was antagonized to the same extent by sotalol alone or in combination with phentolamine, suggesting that the effect of

this agonist is mediated only by beta receptor activation (Krell and Patil, 1969). In rabbit hearts, Lands and Brown (1964) suggested that, since order of potencies of catecholamines were different, production of the rate and force responses are mediated by different receptors. Experiments by Fenyvesi and Hadhazy (1973) demonstrated that in the dog heart-lung preparation, propranolol selectively antagonized the chronotropic effect of isoproterenol. Similar results were observed in humans (Robinson et al., 1973). This hypothesis however, is not supported by data obtained by Blinks (1967) in guinea-pig and kitten atria, since the antagonizing potency of propranolol was very similar for blockade of the inotropic and chronotropic response produced by isoproterenol. Moreover, Birnbaum et al. (1973) observed that the isomeric potency ratio for enantiomers of isoproterenol in rat atria was similar for production of rate and force responses, suggesting similarity of the beta receptors involved.

Receptor changes have been suggested to occur in some tissues when the temperature is decreased. Kunos and Szentivanyi (1968) observed that phentolamine blocked the inotropic and chronotropic effects of epinephrine in rat hearts at 27° C but not at 37° C. In contrast, pronethalol or propranolol blocked the epinephrine effects at 37° C but not at 27° C. Based on these findings, they concluded that there is a transformation from beta into alpha adrenergic receptors at low temperatures. The concept of a single type of adrenergic receptor that "can exist in the either alpha

or beta configuration" was tested with the use of label adrenergic antagonists. When frog hearts were exposed to ^{3}H-phenoxybenzamine the amount of radioactivity bound to the myocardium was twice as great at 15° C than at 25° C. Opposite results were obtained with ^{14}C-propranolol (Kunos et al., 1972). In a more recent communication Kunos et al. (1973) suggested that innervation is necessary for receptor changes induced by low temperatures. They found that after treating rats with 6-OH-dopamine, an agent that selectively damages adrenergic nerve terminals (Tranzer and Thoenen, 1968), phenoxybenzamine did not significantly alter the cardiac response to norepinephrine at either 31° C and 17° C.

The important unitary concept of adrenergic receptors advanced by Kunos and Szentivanyi (1968) has been supported by experiments of other investigators. Buckley and Jordan (1970) demonstrated that in isolated frog heart the order of potencies of catecholamines in increasing cardiac output at 27° C was: isoproterenol > epinephrine > norepinephrine. When the temperature was decreased to 7° C the order of potencies was: norepinephrine > epinephrine > isoproterenol. In guinea-pig atria the order of potencies for the chronotropic effect of catecholamines at 38° C was: isoproterenol > epinephrine > norepinephrine. At 25° C, however, the order of potencies was reversed to: norepinephrine > epinephrine > isoproterenol (Broadley, 1972). In contrast, the order of potencies for the inotropic effects of catecholamines remained the same at 38° C or 25° C (Broadley, 1972).

Furthermore, the potency of practolol in antagonizing the inotropic effect of isoproterenol in guinea-pig atria was found to be the same at 42° C or 25° C (Reinhardt et al., 1972). Therefore, the suggestion that temperature alters the type of adrenergic receptor in heart tissue requires further investigation.

The beta adrenergic receptors have been suggested to be associated with adenylate cyclase (Robison et al., 1971). When this enzyme is activated by catecholamines, there is an increase in the cellular levels of cyclic AMP, the so called "second messenger". Cyclic AMP is broken down to 5' AMP by the enzyme cyclic 3',5'-AMP phosphodiesterase (Butcher and Sutherland, 1962). Cyclic AMP has been implicated in the production of chronotropic and inotropic effects of catecholamines in the heart (Oye et al., 1964; Robison et al., 1965). However, when applied directly to isolated hearts, cyclic AMP can not be demonstrated to produce positive inotropic and chronotropic effects (Robison et al., 1971). The lack of effect of cyclic AMP has been explained on the basis of its inability to penetrate cell membranes (Robison et al., 1971).

It seems possible that the increased sensitivity of heart tissue to catecholamines at low temperatures could be due to a number of alterations in the cyclic AMP system. For instance, reduction of phosphodiesterase activity could result in increased cyclic AMP levels per unit stimulus. Relevant to these considerations is the observation by Marcus et al. (1971) that the sensitivity of isolated cat papillary

muscle to the inotropic effect of glucagon is greater at 30° C than at 37° C. Glucagon has been shown to increase both the levels of cyclic AMP and the inotropic response of intact rat heart (Mayer et al., 1970). In addition, it has been reported that cyclic AMP penetrates cell membranes more readily at low temperatures (Krause and Wollenberger, 1968).

The increased sensitivity to catecholamines at low temperatures could also be due to ionic changes. In heart tissue, contraction is directly associated with calcium movements. Each contraction is brought about by calcium released from the sarcotubular system. This calcium in turn activates an ATPase closely associated with the contractile myofibrils (Ashley, 1971; Schaper et al., 1972). Thus, intracellular calcium appears to be an important link between stimulus and contraction. Langer and Brody (1968) demonstrated that in rabbit ventricular muscle the intracellular calcium increased at 20° C. Furthermore, catecholamines increase intracellular levels of calcium (Nayler, 1967; Dhalla and Braxton, 1968; Carrier et al., 1973). It is then possible that the increased tissue sensitivity may reflect an additive effect of low temperature and catecholamines on calcium movements.

Statement of the problem: It is now well recognized that low temperature sensitizes the myocardium to catecholamines. Despite considerable research in this area, the mechanisms responsible for TDS have not been firmly established. Furthermore, considerable discrepancy exists as to

which is the primary factor involved in the low temperature-induced supersensitivity. Results obtained with norepinephrine and epinephrine are difficult to interpret. These amines are not only neuronally and extraneuronally taken up but are also metabolically degraded by MAO and COMT. Isoproterenol is an adrenergic amine which is not neuronally taken up and is not metabolized by MAO. Consequently, the use of isoproterenol would be more appropriate to study TDS since complications arising from the influence of neuronal uptake and MAO metabolism are practically eliminated.

The classification of adrenergic receptors into alpha and beta receptors postulated by Ahlquist (1948) is widely accepted. His classification was based on the order of potencies of different catecholamines to elicit a response in different tissues. Following the same criteria, different investigators have proposed that at low temperature the adrenergic receptors of heart tissue are transformed from beta into alpha (Kunos and Szentivanyi, 1968). More recently it has been found that in frog heart and guinea-pig atria (inotropic and chronotropic responses) norepinephrine is more potent than isoproterenol at low temperatures (Buckley and Jordan, 1970; Broadley, 1972). Considering the data (see introduction) presented to support the theory, it is observed that none of those investigators followed the criteria outlined by Furchgott (1967; 1970; 1972) to study the receptors involved in a pharmacological response. Ideally, all processes influencing the concentration of agonist at the

receptor should be eliminated. The concentration of norepinephrine and epinephrine at the area of the receptors is influenced by the neuronal uptake process. In rat heart and mouse atria, the affinity of neuronal uptake is greater for norepinephrine than for epinephrine (Iversen, 1967; Sachs, 1970). Because of this differential affinity the relative potencies of these adrenergic amines is not the same in the presence and absence of neuronal uptake. Therefore, changes in the order of potencies do not necessarily indicate a change in receptors.

Results from catecholamines in the absence of neuronal uptake are expected to give better indication of a change of receptors produced by low temperature. Furthermore, the use of antagonists which do not have to rely on production of a response is a better approach to study receptors. Agonists, on the other hand, in addition to combining with receptors, have to produce a response. In view of these considerations, the possibility that low temperature transforms adrenergic receptors is studied in mouse atria with the use of relative potencies of catecholamines, in the absence of neuronal uptake. Different types of antagonists are also used. Additional experiments were designed to study the chronotropic response to cocaine at different temperatures.

The specific experimental goals may be outlined as follows:

1) To determine the effect of low temperature on the sensitivity of isolated atria to catecholamines.

2) To determine relative potencies of (±)-isoproterenol, (-)-norepinephrine and (-)-epinephrine at 37° C and 26° C.

3) To determine pA_2 values for several beta adrenergic antagonists at 37° C and 26° C.

Under proper experimental conditions, if isolated atria are more sensitive at low temperature, the potency of catecholamines should increase. If beta adrenergic receptors of isolated atria are unchanged at 26° C as compared to 37° C, the relative potencies of catecholamines are expected to be similar. Furthermore, pA_2 values should be similar at each temperature.

MATERIALS AND METHODS

Male rabbits (1.3-1.4 kg), guinea-pigs (0.4-0.8 kg), rats (0.15-0.20 kg), and mice (0.025-0.03 kg) were killed by a blow on the head. The heart was removed and placed in a dissecting bath containing Tyrode's solution. The Tyrode's solution had the following composition: $CaCl_2$-$2H_2O$, 1.36 mM; $MgCl_2$, 0.49 mM; KCl, 2.68 mM; $NaHCO_3$, 11.80 mM; NaCl, 147 mM: NaH_2PO_4-H_2O, 0.36 mM; and dextrose, 5.55 mM. All these chemicals were diluted in deionized water. The salt solution was aerated with 95% oxygen 5% CO_2. The pH of the oxygenated Tyrode's solution was 7.2 at 37° C and 7.15 to 7.18 at 26° C. The bath temperature was maintained constant by using water jacketed baths and a constant temperature regulator-circulator.

Preparation of atria: The atria were dissected free of the ventricles and suspended in a tissue bath containing 100 ml of Tyrode's solution. An initial tension of 1 g was applied to the atria and the tissue was allowed to equilibrate for 45 minutes. The bath fluid was changed three times during this period. The final change was made 15 minutes before any drug was added. The spontaneous atrial contractions were recorded on a physiograph (Narco Bio-systems Inc.) via an isometric force displacement transducer. Atrial rates were read directly from a biotachometer (Narco Bio-systems Inc.). Atrial rate after 65 minutes equilibration at 37° C

averaged 359 $\pm$ 17 (S.E.M., n = 7). After 90 minutes the average rate decreased to 312 $\pm$ 7 (S.E.M., n = 15). However, regardless of the length of the initial equilibration period, the tissue sensitivity to the chronotropic effect of isoproterenol remained the same. At 26° C atrial rates remained at a more stable base line.

Evaluation of agonist-induced effects: Cumulative dose-response curves were obtained at either 37° C or 26° C by increasing the agonist concentration by a factor of 3 while the previous concentration remained with the tissue (Van Rossum, 1963). In some experiments the agonist concentration was increased every two minutes and the atrial response determined between 90 and 120 seconds. In others the agonist concentration was increased when the maximum response to the previous dose was attained. In this case the time required to obtain each dose-response curve was approximately 18 minutes. Since norepinephrine and epinephrine are taken up by adrenergic nerve terminals (Iversen, 1967), curves for these amines were obtained in the presence of cocaine to block neuronal uptake.

The results were expressed as per cent of the maximum positive chronotropic response obtained with each agonist. The percent response was calculated according to the following formula:

$$\text{Atrial rate } (\%) = \frac{\text{Observed rate - control rate}}{\text{Maximum rate - control rate}} \times 100$$

The concentration required to produce 50% of the maximum response was determined graphically for each experiment and the effect of agonist evaluated and compared using pD_2 values. The pD_2 value is the negative log of the molar concentration of an agonist that results in production of 50% of the maximum response elicited by that agonist (Ariëns and Van Rossum, 1957).

Evaluation of antagonists: All antagonists were allowed to interact with the tissue for 45 minutes before beginning cumulative addition of the agonist. This incubation period is usually sufficient for development of maximum beta blockade (McInery et al., 1965). The same time period was used for antagonists other than beta receptor blockers, even though antagonism from these compounds could be obtained with shorter incubation periods. When phenoxybenzamine was used, it was incubated with the tissue at 37° C. The alpha blocker was then washed out and the bath temperature decreased to 26° C.

Tropolone, 1×10^{-5} M, was used to block COMT. Experiments in which the tissue was incubated with tropolone and an antagonist, the COMT inhibitor was added 5 minutes before addition of the antagonist so that the total incubation period was 50 minutes for tropolone and 45 minutes for the antagonist.

Evaluation of the effects of cocaine: The effect of

cocaine was determined in mouse atria at 37° C and 26° C. A single dose of cocaine was tested in each tissue. The atrial rate observed after addition of this agent was recorded at different time intervals. Some tissues were exposed to tropolone for 30 minutes prior to addition of cocaine.

Analysis of data: To analyze slopes of dose-response curves, the data for each individual experiment were transformed into probit units and the slope determined according to the following formula:

$$\text{Slope} = \frac{5.842 - 4.158}{\text{Log ED}_{p5.842} - \text{Log ED}_{p4.158}}$$

Where 5.842 and 4.158 are the probit values of 80% and 20% response respectively.

Dose ratios were obtained as:

Antilog (pD_2 without antagonist - pD_2 with antagonist).

The beta adrenergic receptor antagonist were analyzed according to the pA_2 method of Arunlakshana and Schild (1959). The pA_2 is the negative log of the molar concentration of antagonist that produces a 2-fold shift to the right of the agonist dose-response curve. The pA_2 value is a measure of the potency of the antagonist and may be used to differentiate receptors. For competitive antagonism the following relation is given:

$$\text{Log } (x - 1) = \log \frac{1}{K_B} - npA_x$$

Where x = dose ratio, K_B = dissociation constant of the antagonist for the receptor, pA_x = negative log of the antagonist concentration and n is a constant equal to the slope of the regression line. The slope n can be obtained by plotting the log (dose ratio - 1) vs the negative log molar concentration of the antagonist (pA_x). The theoretical value of n should be 1 for competitive antagonism. Since the log (2 - 1) is zero, the intercept of the regression line with the abscissa gives the pA_2 value.

All points obtained by plotting log (dose ratio - 1) vs the negative log molar concentration of the antagonist were connected visually. Regression coefficients were obtained using linear regression equations and analysis of variance was performed according to Steel and Torrie (1960). All other statistical analysis were performed using Student's t test for unpaired observations. A p value of < 0.05 was regarded as statistically significant.

Preparation of drugs: Stock solutions of agonist were prepared in 0.01 N HCl. Dilutions were made from 10^{-2} M stock solutions with 0.01 N HCl. All solutions were stored at 2^{0} C and protected from light. Propranolol solutions were made every 4 days in 0.9% saline. All other agents were made daily in 0.9% saline with 0.05% sodium metabisulfite. Drugs:

(-)-isoproterenol bitartrate (Winthrop Laboratories); (±)-isoproterenol HCl (Mann Research Laboratories, Inc.); (-)-norepinephrine bitartrate (Boehringer Ingelheim); (-)-epinephrine bitartrate (Boehringer Ingelheim); (-)-soterenol HCl (Mead Johnson); Nylidrin HCl (U.S. Vitamin and Pharmaceutical Corp.); phentolamine HCl (Ciba); phenoxybenzamine HCl (Smith Kline & French Laboratories); atropine sulphate (Mallinckrodt Chemical Works); (±)-sotalol (Mead Johnson); (±)-propranolol HCl (Ayerst); cocaine (Mallinckrodt Chemical Works); tropolone (Regis Chemical Co.).

RESULTS

Effect of bath temperature on the sensitivity of isolated rabbit atria to the chronotropic effect of (+)-norepinephrine

To determine the effects of alteration of bath temperature on the sensitivity of rabbit atria to norepinephrine, dose-response curves to this agonist were constructed at 37° C and 26° C. The agonist concentration was increased every 2 minutes. Mean pD_2 values (Table 1) obtained at the two temperatures differ significantly ($P < 0.02$). At 26° C norepinephrine is more potent than at 37° C. The pD_2 ratio was 2.65. Experiments performed with rat atria under the same conditions at 26° C gave a mean pD_2 value of 6.42 ± 0.20 ($n = 6$). This value is quite similar to that obtained by Oppermann (1970). Comparing the pD_2 values obtained from rat atria with those obtained with rabbit atria at 26° C (6.42 for rat vs 5.63 for rabbit), it is apparent that rabbit atria are less sensitive to the chronotropic effects at this temperature.

The observation that low temperature increases the sensitivity of rabbit atria to the chronotropic effect of norepinephrine is in direct contrast to observations by Oppermann (1970) and Booker (1960) who found subsensitivity rather than supersensitivity. The reason for this discrepancy is presently undetermined.

Table 1

Effect of temperature on the sensitivity of rabbit atria to the chronotropic effect of (±)-norepinephrine

Agonist	Atrial rate (beats/min. ± S.E.M.)				pD_2 ± S.E.M.		Ratio[a]	p[b]
	37° C		26° C					
	Initial	Maximum	Initial	Maximum	37° C	26° C		
(±)-Norepinephrine	177 ± 14	328 ± 26	79 ± 3	122 ± 2	5.21 ± 0.03 (4)[c]	5.63 ± 0.11 (8)[c]	2.65	<0.02

[a]Ratio = Antilog (pD_2 26° C - pD_2 37° C).

[b]Level of significance between mean pD_2 values as determined by Student's t test.

[c]Number of observations.

Initial rates were determined before addition of the agonist.

Maximum rates were the highest rate produced by cumulative addition of the agonist.

Effect of bath temperature on the sensitivity of isolated rabbit, rat, and mouse atria to the chronotropic effect of (-)-isoproterenol

The effect of temperature on the potency of isoproterenol was evaluated in rabbit, rat and mouse atria. Table 2 shows the pD_2 values obtained at 37° C and 26° C. The pD_2 values are significantly greater at 26° C in all species. This indicates that atria are more sensitive to the effects of isoproterenol at low temperature. It is also observed that rat atria show the greatest degree of change in sensitivity with temperature while rabbit atria show the least.

Cocaine, 1×10^{-5} M, did not affect the potency of isoproterenol at either 37° C or 26° C in rabbit atria.

Effect of temperature on the sensitivity of isolated mouse atria to the chronotropic effect of catecholamines

In Figure 1 the spontaneous atrial rate is plotted as the per cent of the maximum response produced by each agonist vs the negative log of the molar concentration of the agonist. Data are summarized in Table 3. It was observed that at 26° C the dose-response curves of the three agonists (isoproterenol, norepinephrine, and epinephrine) were shifted to the left. The pD_2 values (Table 3) obtained at 26° C were significantly greater than those obtained at 37° C. The degree of potentiation produced by the 11° C decrease in bath temperature was greatest for isoproterenol and least for norepinephrine. At 37° C the relative potencies of catecholamines were

Table 2

Effect of temperature on the sensitivity of isolated atria to the positive chronotropic effect of (-)-isoproterenol

Species	Atrial rate (beats/min ± S.E.M.)				pD_2 ± S.E.M.		Ratio[a]	p[b]
	37° C		26° C					
	Initial	Maximum	Initial	Maximum	37° C	26° C		
Rabbit	178 ± 13	292 ± 5	73 ± 5	133 ± 3	6.56 ± 0.06 (5)[c] *6.41 ± 0.09 (4)[c]	7.54 ± 0.05 (6)[c] *7.46 ± 0.12 (4)[c]	9.60	<0.001
Rat	2.71 ± 6	407 ± 14	120 ± 11	204 ± 1	6.49 ± 0.06 (4)[c]	8.21 ± 0.08 (4)[c]	51.76	<0.01
Mouse	341 ± 14	564 ± 19	182 ± 10	284 ± 11	6.52 ± 0.06 (4)[c]	7.79 ± 0.13 (4)[c]	18.62	<0.01

[a] Ratio = Antilog (pD_2 26° C - pD_2 37° C).

[b] Level of significance between mean pD_2 values as determined by Student's t test.

Table 2 (continued)

[c]Number of observations.

*Tissues were exposed to cocaine, 1×10^{-5} M.

Initial rates were determined before addition of the agonist.

Maximum rates were the highest rate produced by cumulative addition of the agonist.

Figure 1. Mean cumulative dose-response curves for (±)-isoproterenol, (-)-norepinephrine and (-)-epinephrine obtained in mouse atria at 37° C and 26° C. Curves for norepinephrine and epinephrine were obtained in the presence of cocaine, 1×10^{-5} M. Numbers in parentheses are number of observations. Vertical lines indicate S.E.M.

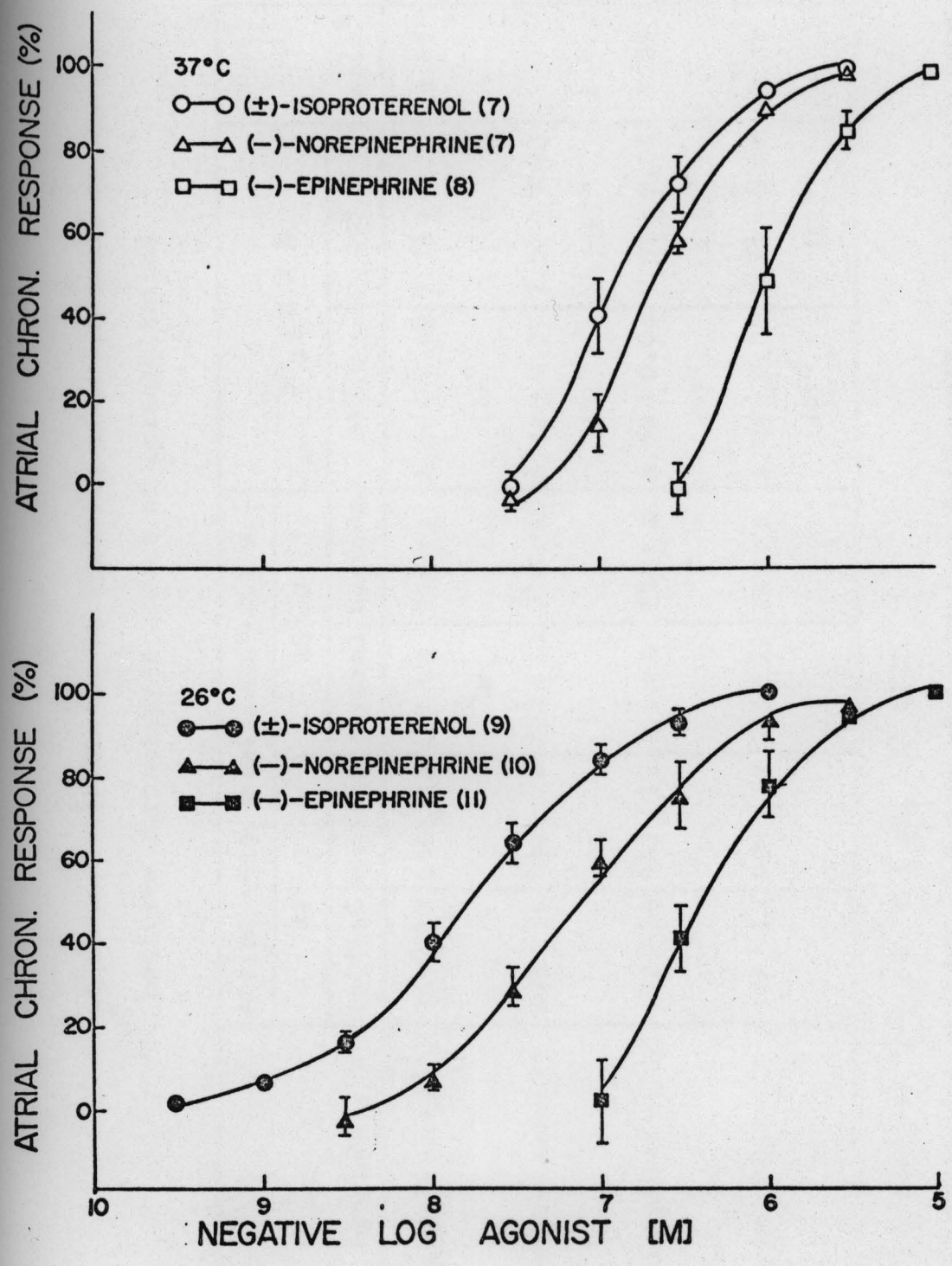

37°C
(±)-ISOPROTERENOL (7)
(−)-NOREPINEPHRINE (7)
(−)-EPINEPHRINE (8)
ATRIAL CHRON. RESPONSE (%)
100
80
60
40
20
0
26°C
(±)-ISOPROTERENOL (9)
(−)-NOREPINEPHRINE (10)
(−)-EPINEPHRINE (11)
ATRIAL CHRON. RESPONSE (%)
100
80
60
40
20
0
10
9
8
7
6
5
NEGATIVE LOG AGONIST [M]

Table 3

Effect of temperature on the sensitivity of mouse atria to the positive chronotropic effect of catecholamines

Agonist	Atrial rate (beats/min ± S.E.M.)				pD_2 ± S.E.M.		Ratio[a]	P[b]
	37° C		26° C					
	Initial	Maximum	Initial	Maximum	37° C	26° C		
(±)-Isoproterenol	359 ± 17	590 ± 14	171 ± 6	288 ± 6	6.80 ± 0.08 (7)[c]	7.74 ± 0.12 (9)[c]	8.65	<0.001
(±)-Isoproterenol*	348 ± 16	574 ± 17	234 ± 10	276 ± 10	6.74 ± 0.07 (7)[c]	7.53 ± 0.11 (8)[c]		
(-)-Norepinephrine*	342 ± 16	583 ± 13	215 ± 9	274 ± 8	6.62 ± 0.04 (7)[c]	7.08 ± 0.05 (10)[c]	2.85	<0.001
(-)-Epinephrine*	391 ± 17	590 ± 13	212 ± 11	249 ± 5	5.94 ± 0.08 (8)[c]	6.42 ± 0.08 (11)[c]	3.02	<0.001

[a] Ratio = Antilog (pD_2 26° C - pD_2 37° C).

[b] Level of significance between mean pD_2 values as determined by Student's t test.

[c] Number of observations.

Table 3 (continued)

*Tissues were exposed to cocaine, 1×10^{-5} M.

Initial rates were determined before addition of agonists.

Maximum rates were the highest rates produced by each agonist.

isoproterenol 1.32, norepinephrine 1, and epinephrine 0.21, whereas at 26° C the relative potencies were isoproterenol 2.82, norepinephrine 1, and epinephrine 0.22.

It was observed that at 26° C the initial rate was greater in the presence than in the absence of cocaine. At 37° C cocaine did not alter the initial atrial rate. However, the maximum rates produced by the three catecholamines were about the same at each temperature. Cocaine, 1×10^{-5} M, did not alter the pD_2 values of isoproterenol at either 37° C or 26° C.

Effect of COMT inhibition by tropolone on the potency of isoproterenol and slope of isoproterenol dose-response curves at different temperatures

Table 4 shows pD_2 values for isoproterenol obtained in mouse atria in the absence and presence of tropolone. The change in pD_2 values induced by temperature was qualitatively similar in both cases. Exposure of the tissue to tropolone, 1×10^{-5} M, for 50 minutes, reduced the ratio of pD_2 values from 10.70 to 4.65. With this concentration of tropolone there was still a significant difference between pD_2 values for isoproterenol at the two temperatures. This suggested that temperature may still influence the potency of isoproterenol. However, when the tissue was exposed to a higher concentration of tropolone, 1×10^{-4} M, for 30 minutes, the effect of temperature was completely abolished.

Slope values were calculated for those curves obtained

Table 4

Effect of temperature and tropolone on the potency of isoproterenol and slope of isoproterenol dose-response curves in mouse atria

Agonist	$pD_2 \pm$ S.E.M.		Ratio[a]	P[b]	Slope (probit/log dose)		P[b]
	37° C	26° C			37° C	26° C	
(±)-Isoproterenol	6.73 ± 0.09 (7)[c]	7.76 ± 0.09 (6)[c]	10.70	<0.001	2.61 ± 0.23	1.41 ± 0.10	<0.001
(±)-Isoproterenol with tropolone 1×10^{-5} M	8.20 ± 0.06 (5)[c]	8.85 ± 0.14 (6)[c]	4.65	<0.01	1.28 ± 0.13	1.11 ± 0.13	>0.05
(-)-Isoproterenol	7.37 ± 0.13 (5)[c]	8.59 ± 0.13 (8)[c]	16.61	<0.001			
(-)-Isoproterenol with tropolone 1×10^{-4} M	9.00 ± 0.08 (6)[c]	9.34 ± 0.27 (6)[c]	2.18	>0.05			

[a] Ratio = Antilog (pD_2 26° C - pD_2 37° C).

[b] Level of significance between mean pD_2 values; between mean slope values as determined by Student's t test.

[c] Number of observations.

obtained with racemic isoproterenol. At 26° C the slope value was 1.41. This value was significantly different from the slope (2.61) obtained at 37° C. Tropolone decreased the slope of the dose-response curve from 2.61 to 1.28 at 37° C. However, in the presence of tropolone, slope values at 37° C and 26° C did not differ significantly. Slope values were also calculated from mean norepinephrine dose-response curves. For this amine the slope values were 2.46 and 1.27 at 37° C and 26° C, respectively.

Effect of temperature on the sensitivity of isolated mouse atria to the chronotropic effect of nylidrin and (-)-soterenol

Nylidrin and soterenol are non-catechol beta adrenergic receptor agonists. The effect of temperature on the sensitivity of mouse atria to these compounds was studied at 37° C and 26° C. Cumulative dose-response curves were obtained by increasing the agonist concentration every 2 minutes in the case of soterenol, and after the maximum response to each dose was attained in the case of nylidrin. For soterenol (Table 5) there was no significant change in the pD_2 values at either 37° C or 26° C. Similarly, the 11° C decrease in bath temperature did not alter the potency of nylidrin.

Effect of bath temperature on the potency of different antagonists

The effect of temperature on the potency of different antagonists was evaluated in mouse atria at 37° C and 26° C.

Table 5

Effect of temperature on the sensitivity of mouse atria to the positive chronotropic effect of non-catechol beta adrenergic agonists

Agonist	Atrial rate (beats/min ± S.E.M.)				pD_2 ± S.E.M.		Ratio[a]	P[b]
	37° C		26° C					
	Initial	Maximum	Initial	Maximum	37° C	26° C		
(-)-Soterenol	408 ± 15	531 ± 14	173 ± 11	229 ± 12	6.53 ± 0.10 (6)[c]	6.46 ± 0.15 (6)[c]	0.85	>0.05
Nylidrin	319 ± 10	475 ± 12	150 ± 10	222 ± 6	6.40 ± 0.08 (6)[c]	6.15 ± 0.14 (6)[c]	0.60	>0.05

[a] Ratio = Antilog (pD_2 26° C - pD_2 37° C).

[b] Level of significance between mean pD_2 values as determined by Student's t test.

[c] Number of observations.

Initial rates were determined before addition of the agonist.

Maximum rates were the highest rate produced by each agonist.

For the series of experiments to be described, (-)-isoproterenol concentrations were added every 2 minutes to construct the dose-response curve. When racemic isoproterenol was used, concentrations were increased when the maximum response to each dose was reached.

Figures 2, 3, and 4 show the atrial response to isoproterenol at 37° C and 26° C in the absence and presence of atropine, phentolamine and sotalol. Data are summarized in Table 6. None of these antagonists altered the maximum rate produced by isoproterenol at either temperature. The potency of antagonists is definitely influenced by temperature. Atropine, 1×10^{-8} M, had no effect on the sensitivity of atria to the chronotropic effect of isoproterenol at 37° C. In contrast, an 11° C decrease in bath temperature unmasked blocking properties of atropine. The dose ratio for atropine obtained at 26° C was 7.25. However, this antagonism exerted by atropine was not dose dependent. A ten-fold increase in atropine concentration did not result in an increase of the dose ratio. Instead, the dose ratio decreased from 7.25 to 3.40.

Phentolamine shifted the dose-response curve to the right at 37° C. The dose ratio was 4. However, at 26° C the same concentration of phentolamine had no significant effect on the potency of isoproterenol.

Since the blocking effects of phentolamine were unexpected, it was decided to investigate effects of another alpha adrenergic antagonist, phenoxybenzamine. This agent

Figure 2. Mean cumulative dose-response curves for the chronotropic effect of (-)-isoproterenol obtained in mouse atria at 37^{0} C and 26^{0} C in the absence and presence of atropine, 1×10^{-8} M. Numbers in parentheses are number of observations. Vertical lines indicate S.E.M.

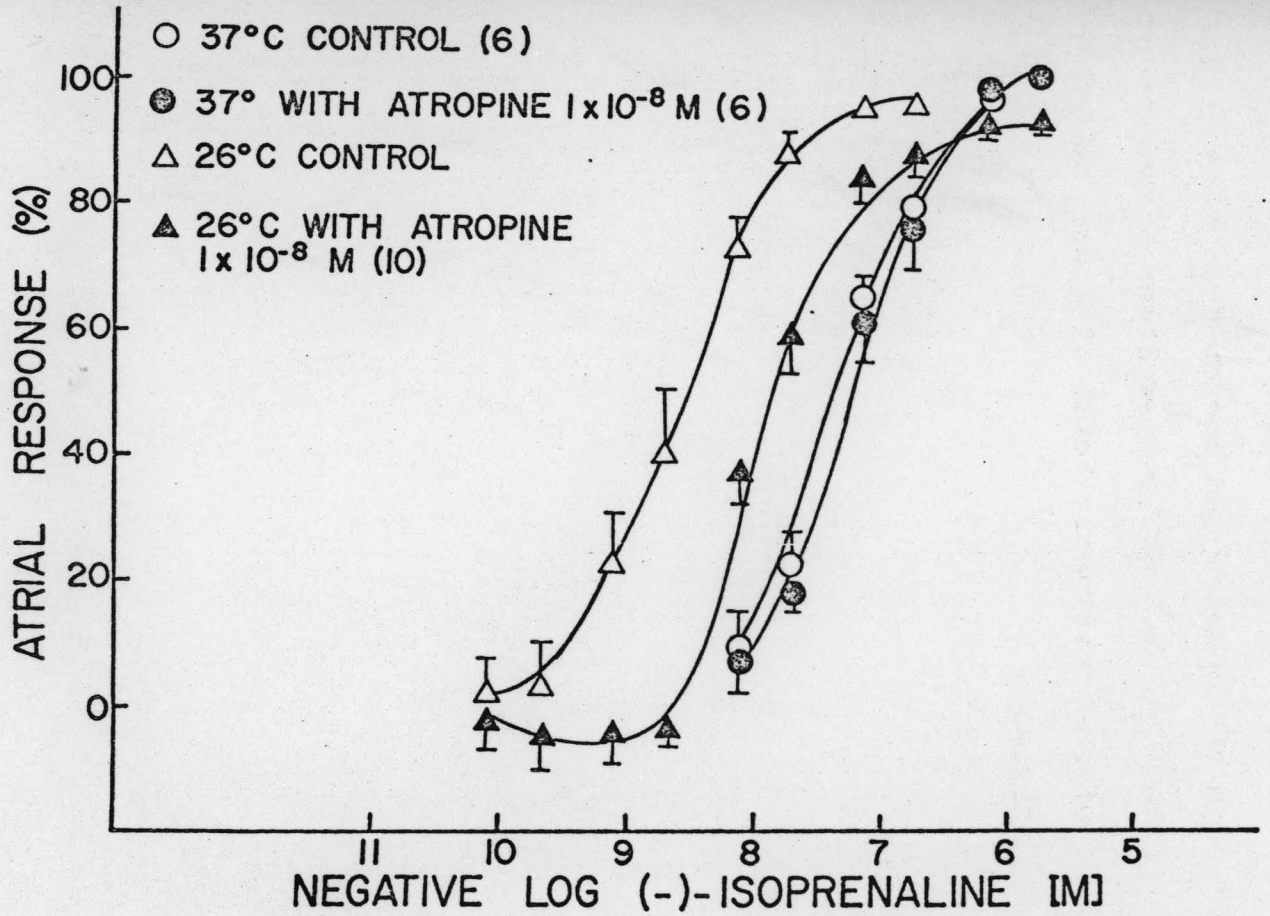

○ 37°C CONTROL (6)
● 37° WITH ATROPINE 1 x 10⁻⁸ M (6)
△ 26°C CONTROL
▲ 26°C WITH ATROPINE 1 x 10⁻⁸ M (10)
ATRIAL RESPONSE (%)
100
80
60
40
20
0
11
10
9
8
7
6
5
NEGATIVE LOG (-)-ISOPRENALINE [M]

Figure 3. Mean cumulative dose-response curves for the chronotropic effect of (-)-isoproterenol obtained in mouse atria at 37^{0} C and 26^{0} C in the absence and presence of phentolamine, 1×10^{-7} M. Numbers in parentheses are number of observations. Vertical lines indicate S.E.M.

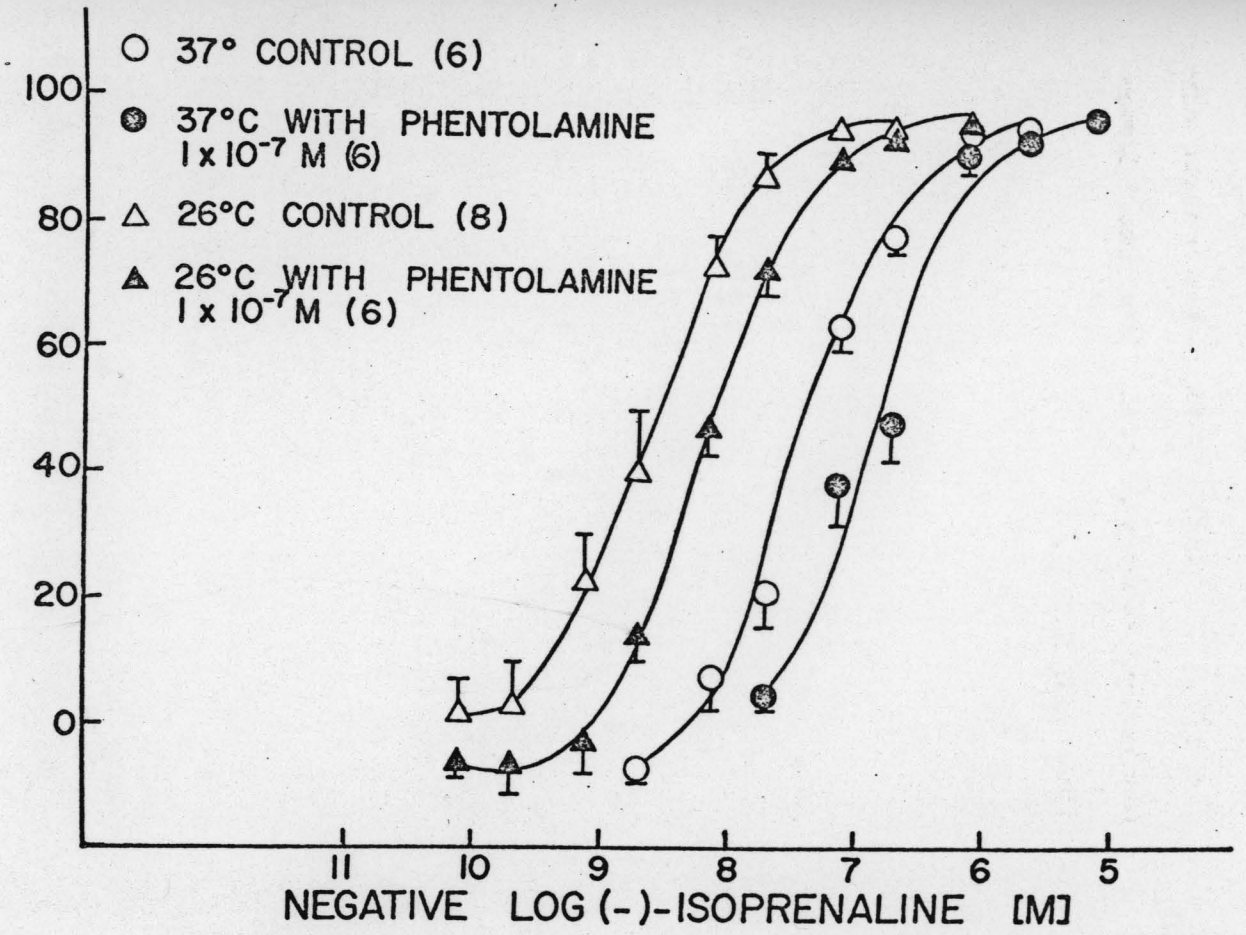

37° CONTROL (6)
37°C WITH PHENTOLAMINE 1 x 10⁻⁷ M (6)
26°C CONTROL (8)
26°C WITH PHENTOLAMINE 1 x 10⁻⁷ M (6)
100
80
60
40
20
0
11
10
9
8
7
6
5
NEGATIVE LOG (-)-ISOPRENALINE [M]

Figure 4. Mean cumulative dose-response curves for the chronotropic effect of (-)-isoproterenol obtained in mouse atria at 37° C and 26° C in the absence and presence of (±)-sotalol, 1×10^{-6} M. Numbers in parentheses are number of observations. Vertical lines indicate S.E.M.

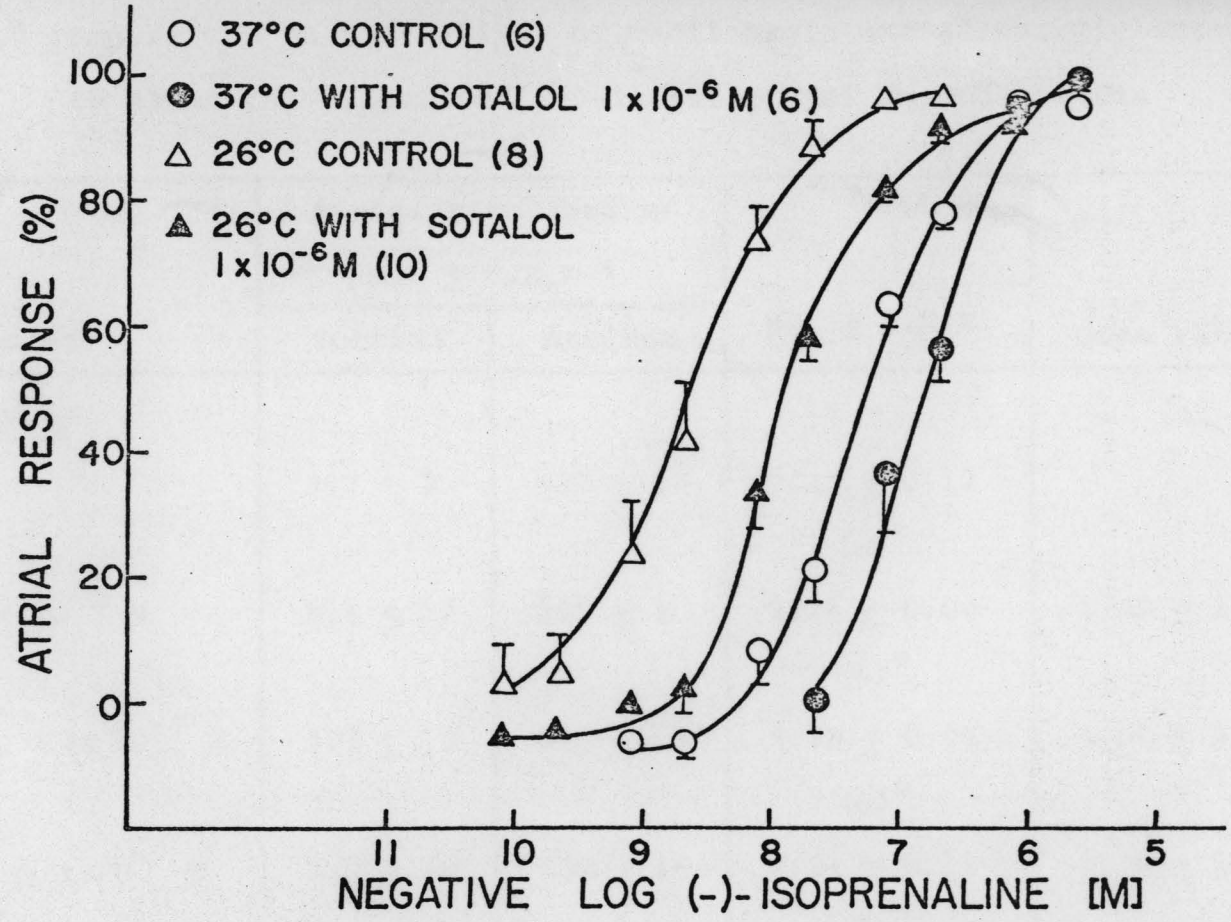

○ 37°C CONTROL (6)
● 37°C WITH SOTALOL 1 x 10⁻⁶ M (6)
△ 26°C CONTROL (8)
▲ 26°C WITH SOTALOL 1 x 10⁻⁶ M (10)
ATRIAL RESPONSE (%)
100
80
60
40
20
0
11
10
9
8
7
6
5
NEGATIVE LOG (-)-ISOPRENALINE [M]

Table 6

Effect of temperature on the ability of cholinergic and adrenergic antagonists to alter the effects of (-)-isoproterenol on mouse atria

Treatment	Atrial rate (beats/min ± S.E.M.)		pD_2 ± S.E.M.	Dose ratio[a]	p[b]
	Initial	Maximum			
At 37° C					
None	357 ± 7	587 ± 10	7.38 ± 0.12 (6)[c]		
Atropine, 1×10^{-8} M	316 ± 12	585 ± 8	7.21 ± 0.04 (6)[c]	1.48 ± 1.37	>0.05
Phentolamine, 1×10^{-7} M	329 ± 19	572 ± 15	6.78 ± 0.06 (6)[c]	4.00 ± 1.39	<0.05
(±)-Sotalol, 1×10^{-6} M	330 ± 18	598 ± 16	6.91 ± 0.11 (6)[c]	2.95 ± 1.50	<0.05
At 26° C					
None	167 ± 9	273 ± 8	8.59 ± 0.11 (8)[c]		

Table 6 (continued)

Treatment	Atrial rate (beats/min ± S.E.M.)		pD_2 ± S.E.M.	Dose ratio[a]	P[b]
	Initial	Maximum			
Atropine, 1×10^{-8} M	189 ± 11	321 ± 8	7.73 ± 0.10 (10)[c]	7.25 ± 1.46	<0.001
Atropine, 1×10^{-7} M	178 ± 6	277 ± 5	8.06 ± 0.10 (14)[c]	3.40 ± 1.46	<0.01
Phentolamine, 1×10^{-7} M	167 ± 6	292 ± 8	8.16 ± 0.10 (6)[c]	2.69 ± 1.35	>0.05
(±)-Sotalol, 1×10^{-6} M	140 ± 9	277 ± 10	7.59 ± 0.07 (10)[c]	10.00 ± 1.40	<0.001

[a]Dose ratio = Antilog (pD_2 without antagonist - pD_2 with antagonist).

[b]Level of significance between mean pD_2 values as determined by Student's t test.

[c]Number of observations.

Initial rates were determined before addition of the agonist.

Maximum rates were the highest rate produced by the agonist.

binds covalently to alpha receptors, rendering them unavailable for combination with other drugs. Table 7 shows the results from these experiments. Under these experimental conditions phenoxybenzamine did not alter the potency of isoproterenol.

Sotalol, 1×10^{-6} M, (Figure 4, Table 6) shifted the dose-response curve to the right at both 37° C and 26° C. At 37° C sotalol produced a dose ratio of 2.95. An 11° C decrease in bath temperature significantly increased the potency of sotalol. At 26° C the dose ratio increased to 10.

The effect of temperature on the potency of sotalol was also studied using (±)-isoproterenol as the agonist. At 37° C (Figure 6) sotalol, 1×10^{-5} M, significantly shifted the dose-response curve to the right. Increasing the sotalol concentration to 1×10^{-4} M resulted in a further shift of the dose-response curve. In addition Figure 5 illustrates that at 26° C the potency of sotalol is increased. A plot of log (dose ratio - 1) versus log molar antagonist concentration is presented in Figure 6. It is observed that the pA_2 value for sotalol increased from 5.79 at 37° C to 6.48 at 26° C. There is however, no apparent change in the slope of the regression lines at either 37° C or 26° C.

Experiments were also performed with nylidrin in the absence and presence of sotalol, 1×10^{-5} M, at 37° C and 26° C. Since nylidrin was not a full agonist, the $ED30_s$ rather than the $ED50_s$ were used to make comparisons. The ED30 was calculated as the concentration of nylidrin

Table 7

Effect of phenoxybenzamine on the sensitivity of isolated mouse atria to the positive chronotropic effect of (±)-isoproterenol

Treatment	Atrial rate (beats/min ± S.E.M.)		pD_2 ± S.E.M.	Dose ratio[a]	P[b]
	Initial	Maximum			
None	113 ± 9	249 ± 6	7.76 ± 0.05 (9)[c]		
Phenoxybenzamine*	139 ± 7	258 ± 8	7.68 ± 0.04 (8)[c]	1.21 ± 1.15	>0.05

*Tissues incubated with phenoxybenzamine at 37° C followed by a decrease in temperature to 26° C.

[a]Dose ratio = Antilog (pD_2 without antagonist - pD_2 with antagonist).

[b]Level of significance between mean pD_2 values as determined by Student's t test.

[c]Number of observations.

Initial rates were determined at 26° C before addition of the agonist.

Maximum rates were the highest rate produced by the agonist.

Figure 5. Mean cumulative dose-response curves for (±)-isoproterenol obtained in mouse atria at 37° C and 26° C in the absence and presence of (±)-sotalol, 1×10^{-5} M - 1×10^{-4} M. Numbers in parentheses are number of observations. Vertical lines indicate S.E.M.

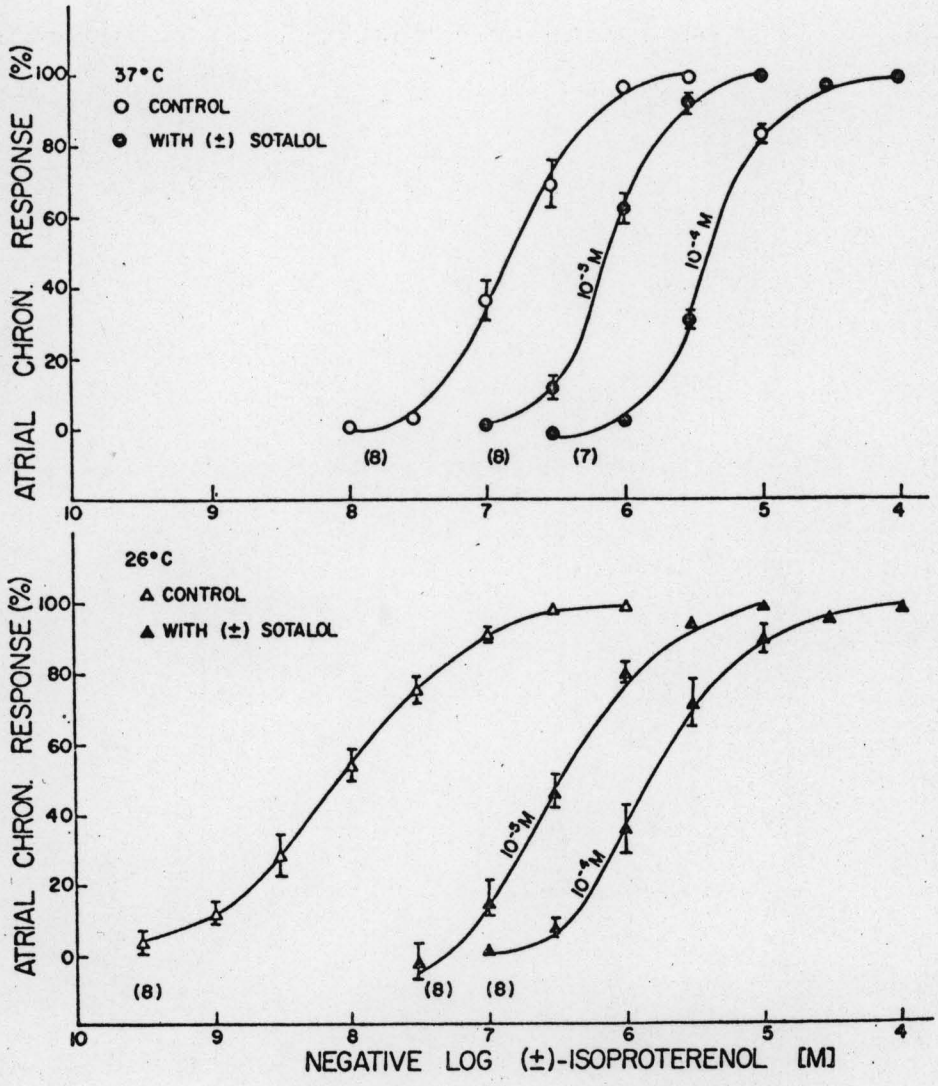
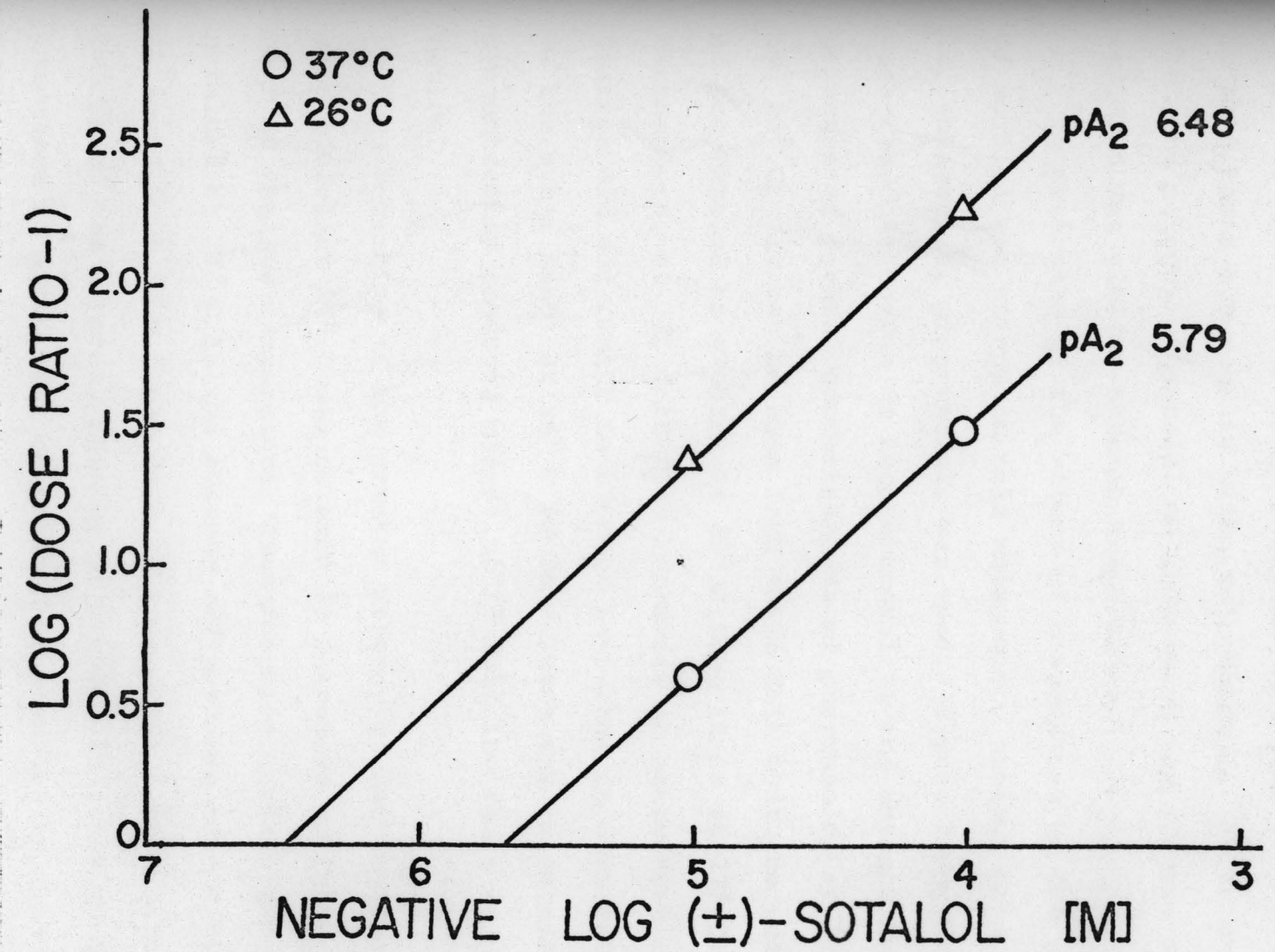

37°C
CONTROL
WITH (±) SOTALOL
ATRIAL CHRON. RESPONSE (%)
10⁻³M
10⁻⁴M
(8)
(8)
(7)
26°C
CONTROL
WITH (±) SOTALOL
(8)
(8)
(8)
NEGATIVE LOG (±)-ISOPROTERENOL [M]

Figure 6. Plots of log (dose ratio - 1) vs negative log molar concentration of (±)-sotalol obtained in mouse atria at 37° C and 26° C. Number of observations are the same as in Figure 5.


O 37°C
Δ 26°C
pA2 6.48
pA2 5.79
LOG (DOSE RATIO-1)
2.5
2.0
1.5
1.0
0.5
0
7
6
5
4
3
NEGATIVE LOG (±)-SOTALOL [M]

producing 30% of the maximum response produced by (±)-isoproterenol. Results are presented in Table 8. Included in this table are results with isoproterenol. It is observed that the potency of sotalol to antagonize the effects of nylidrin was similar at each temperature.

To determine if the effect of temperature on the potency of sotalol was unique to this antagonist, another beta receptor antagonist, propranolol, was used. Figure 7 depicts dose-response curves to isoproterenol in the absence and presence of several concentrations of propranolol at 37° C and 26° C. It can be seen from these data that the potency of propranolol is greater at 26° C than it is at 37° C. Furthermore, at 37° C 10-fold increases in antagonist concentration did not result in 10-fold increases in dose ratios. On the other hand, at 26° C 10-fold increases in antagonist concentration produced closer to 10-fold increases in dose ratios.

To determine if the greater potency of both sotalol and propranolol at 26° C was related to decreased activity of COMT at the low temperature, dose-response curves were obtained at 37° C in the absence and presence of tropolone. It is evident from these data (Figure 8) that tropolone increased the apparent potency of propranolol, and 10-fold increases in antagonist concentration resulted in approximately 10-fold increases in dose ratios.

Results from experiments performed at 26° C in the presence of tropolone are presented in Figure 9 (included in

Table 8

Effect of temperature on the ability of (±)-sotalol to antagonize the positive chronotropic effect of (±)-isoproterenol and nylidrin in mouse atria

Agonist	-Log molar concentration with S.E.M. [a]			
	37° C		26° C	
		Dose ratio [b]		Dose ratio [b]
Nylidrin	6.18 ± 0.15 (6)		6.13 ± 0.19 (5)	
Nylidrin + (±)-sotalol, 1×10^{-5} M	5.12 ± 0.14 (5)	12.0 ± 1.65	5.20 ± 0.15 (4)	8.52 ± 1.74
(±)-Isoproterenol	6.79 ± 0.07 (8)		8.03 ± 0.11 (8)	
(±)-Isoproterenol + (±)-sotalol, 1×10^{-5} M	6.05 ± 0.03 (8)	5.01 ± 1.20	6.63 ± 0.11 (8)	25.1 ± 1.43

[a] Values for nylidrin are -Log molar concentration producing 30% of the maximum response produced by isoproterenol. Values for isoproterenol are pD_2 values. Numbers in

Table 8 (continued)

parentheses are number of observations.

[b]Dose ratio = Antilog (pD_2 without antagonist - pD_2 with antagonist).

Figure 7. Mean cumulative dose-response curves for (±)-isoproterenol obtained in mouse atria at 37° C and 26° C in the absence and presence of (±)-propranolol, 1 x 10^{-8} - 10^{-7} - 10^{-6} M. Numbers in parentheses are number of observations. Vertical lines indicate S.E.M.

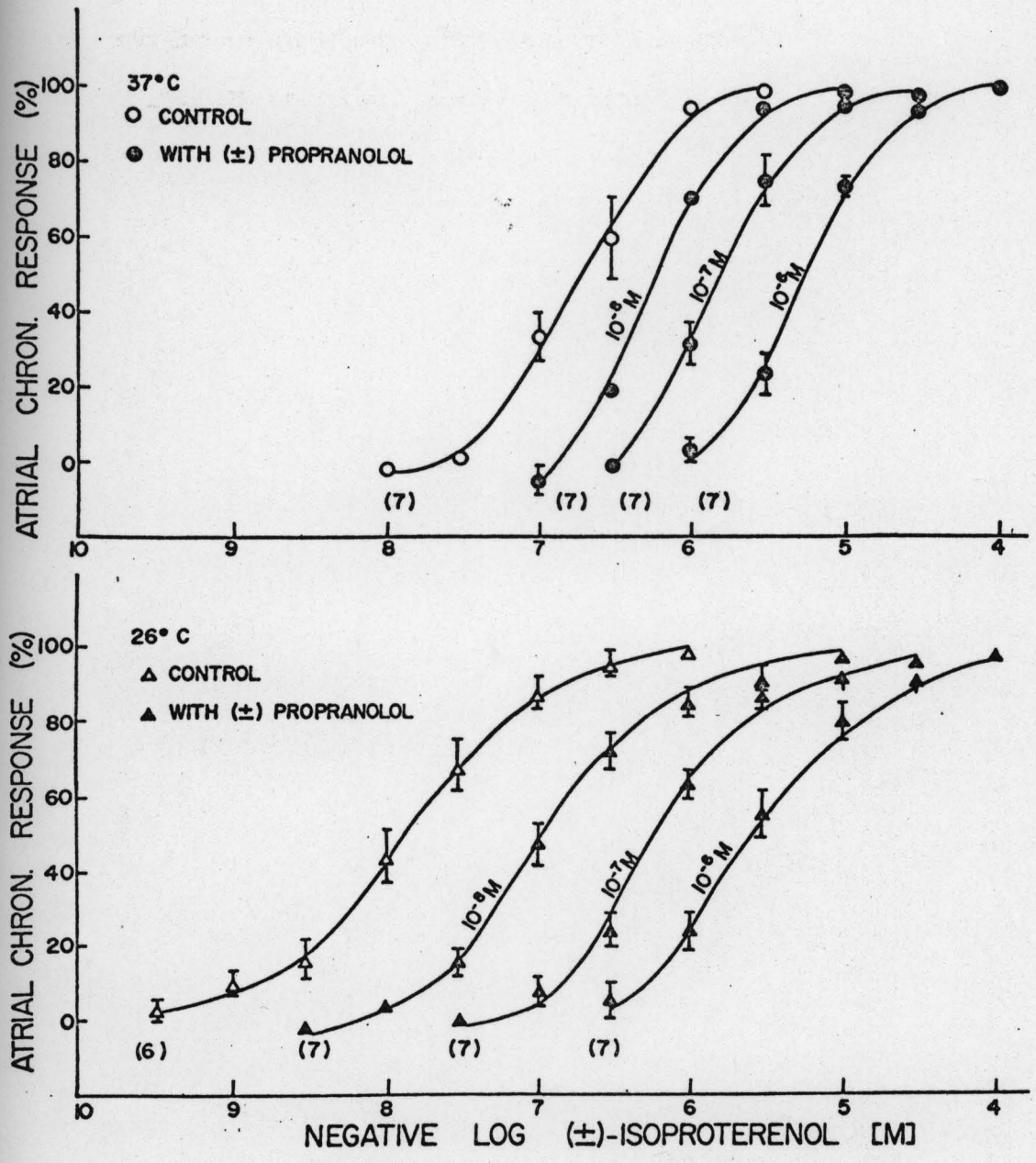

37° C
CONTROL
WITH (±) PROPRANOLOL
ATRIAL CHRON. RESPONSE (%)
10⁻⁸M
10⁻⁷M
10⁻⁶M
(7)
(7)
(7)
(7)
26° C
CONTROL
WITH (±) PROPRANOLOL
ATRIAL CHRON. RESPONSE (%)
10⁻⁸M
10⁻⁷M
10⁻⁶M
(6)
(7)
(7)
(7)
NEGATIVE LOG (±)-ISOPROTERENOL [M]

Figure 8. Mean cumulative dose-response curves for (±)-isoproterenol obtained in mouse atria at 37° C in the absence and presence of (±)-propranolol, $1 \times 10^{-8} - 10^{-7} - 10^{-6}$ M. In the lower panel all curves were obtained in the presence of tropolone, 1×10^{-5} M. Numbers in parentheses are number of observations. Vertical lines indicate S.E.M.

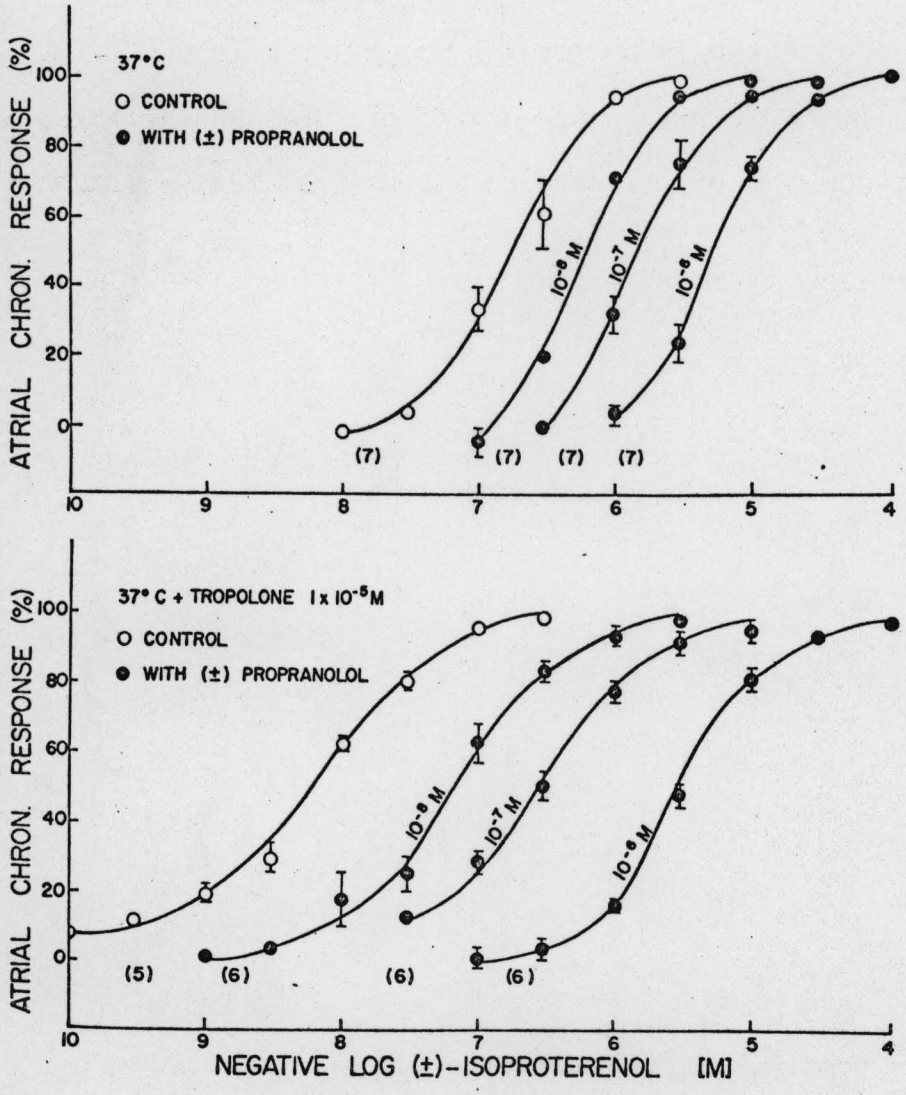
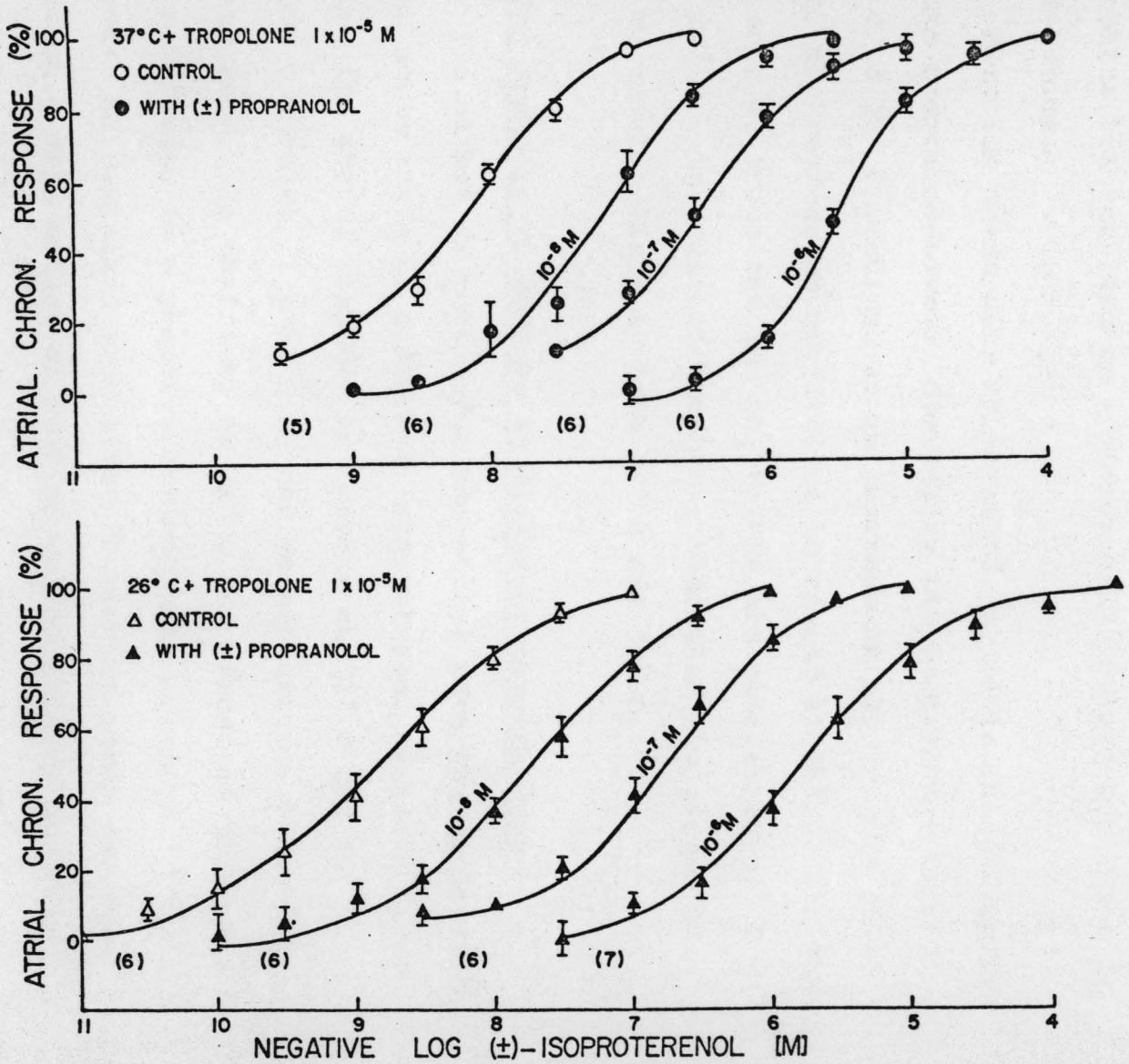

37°C
CONTROL
WITH (±) PROPRANOLOL
ATRIAL CHRON. RESPONSE (%)
100
80
60
40
20
0
10⁻⁸ M
10⁻⁷ M
10⁻⁶ M
(7)
(7)
(7)
(7)
10
9
8
7
6
5
4
37° C + TROPOLONE 1 x 10⁻⁵M
CONTROL
WITH (±) PROPRANOLOL
ATRIAL CHRON. RESPONSE (%)
10⁻⁸ M
10⁻⁷ M
10⁻⁶ M
(5)
(6)
(6)
(6)
NEGATIVE LOG (±)-ISOPROTERENOL [M]

Figure 9. Mean cumulative dose-response curves for (±)-isoproterenol obtained in mouse atria at 37° C and 26° C in the absence and presence of (±)-propranolol, 1 x 10^{-8} - 10^{-7} - 10^{-6} M. All these curves were obtained in the presence of tropolone, 1 x 10^{-5} M. Numbers in parentheses are number of observations. Vertical lines are S.E.M.


37° C + TROPOLONE 1 x 10⁻⁵ M
CONTROL
WITH (±) PROPRANOLOL
ATRIAL CHRON. RESPONSE (%)
10⁻⁸ M
10⁻⁷ M
10⁻⁶ M
(5)
(6)
(6)
(6)
26° C + TROPOLONE 1 x 10⁻⁵M
CONTROL
WITH (±) PROPRANOLOL
(6)
(6)
(6)
(7)
NEGATIVE LOG (±)-ISOPROTERENOL [M]

this figure are the data at 37° C). It is observed that in teh presence of tropolone the apparent potency of propranolol was not increased at 26° C. Ten-fold increases in antagonist concentration resulted in approximately 10-fold increases in dose ratios.

Plotting these data in terms of pA_2 plots the following points can be observed: decreasing the temperature from 37° C to 26° C resulted in an increase in pA_2 (Figure 10). Also, the regression coefficient increased at 26° C. In the presence of tropolone the pA_2 values are similar at 37° C and 26° C. In addition tropolone increased the slopes of the regression lines at both 37° C and 26° C.

Atrial response to cocaine at different temperatures

The effect of cocaine on the atrial rate was determined using mouse atria at different temperatures. Table 9 illustrates the atrial response to cocaine at 37° C and 26° C. At 37° C cocaine, 1×10^{-5} M, had no significant effect on atrial rate. In contrast, at 26° C the effect of cocaine was manifested as a positive chronotropic response. This response is sustained even after 50 minutes after cocaine addition. This effect of cocaine was completely abolished by sotalol 1×10^{-6} M. At 37° C the positive chronotropic effect of cocaine was unmasked by exposing the tissue to tropolone 1×10^{-5} M.

At 26° C an inverse relationship between the initial rates and the response to cocaine was observed (Y = 127.3 -

Figure 10. Plots of log (dose ratio - 1) vs negative log molar concentration of (±)-propranolol obtained in mouse atria at 37° C and 26° C. In the lower panel similar plots of data obtained in the presence of tropolone, 1 x 10^{-5} M. Number of observations are the same as in Figures 8-10. Numbers on regression lines are regression coefficients. Vertical lines indicate S.E.M. When not present, the S.E.M. are within the points.

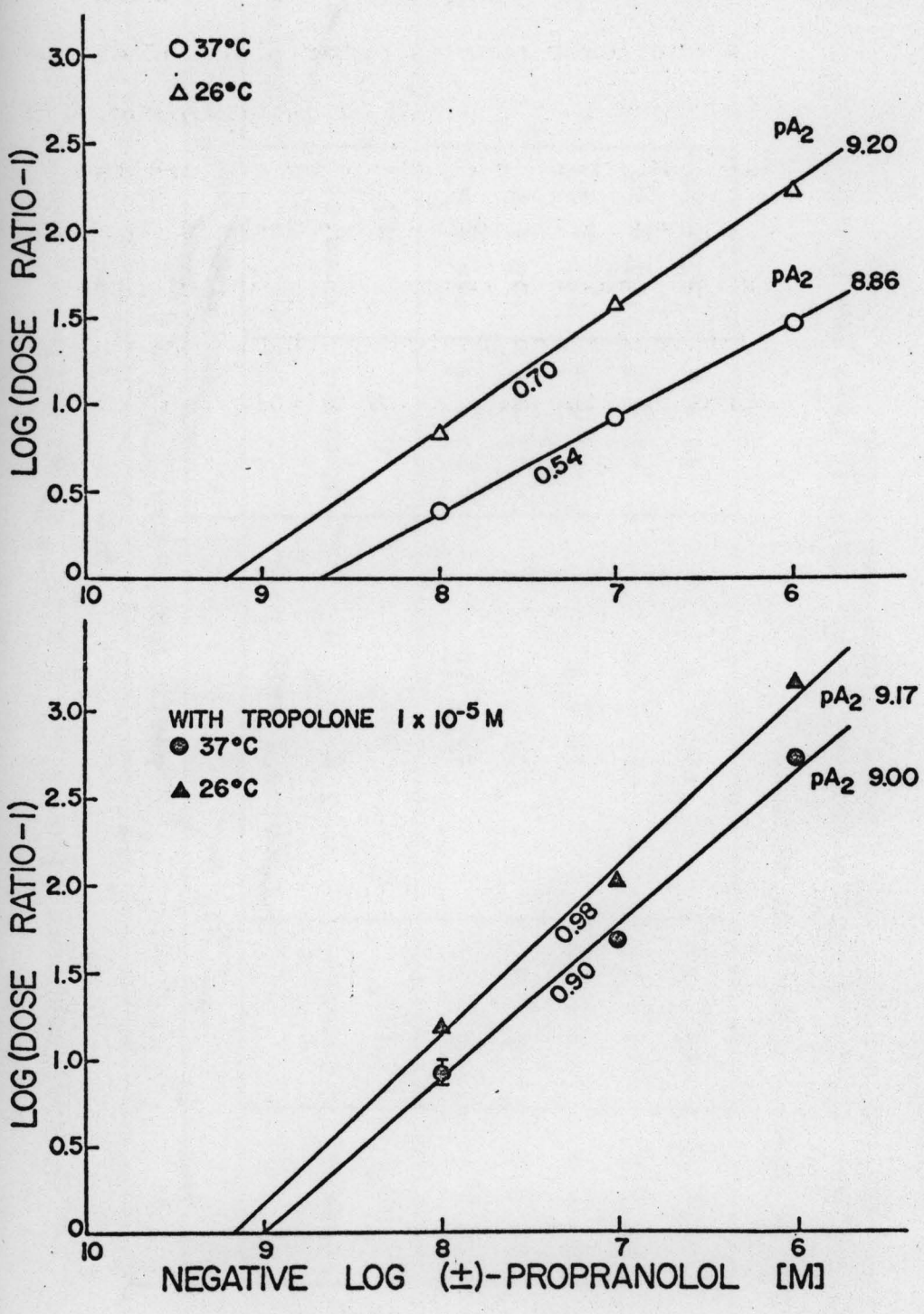

○ 37°C
△ 26°C
pA2 9.20
pA2 8.86
0.70
0.54
LOG (DOSE RATIO-1)
WITH TROPOLONE 1 x 10-5 M
● 37°C
▲ 26°C
pA2 9.17
pA2 9.00
0.98
0.90
NEGATIVE LOG (±)-PROPRANOLOL [M]

Table 9

Atrial response to cocaine, 1×10^{-5} M, at different temperatures

Time (min)[a]	Δrate (beats/min ± S.E.M.)[b]					
	37° C			26° C		
	Control (6)[c]	Cocaine (4)[c]	Cocaine + tropolone, 1×10^{-5} M (3)[c]	Control (4)[c]	Cocaine (6)[c]	Cocaine + sotalol 1×10^{-6} M (6)[c]
10	+5 ± 2	-10 ± 15	-5 ± 16	+3 ± 4	+46 ± 8	-7 ± 4
20	+5 ± 4	-7 ± 6	+25 ± 5	-2 ± 5	+50 ± 8	-7 ± 5
30	-7 ± 6	-16 ± 10	+19 ± 7	-6 ± 6	+57 ± 9	-5 ± 6
40	-9 ± 7	-27 ± 11	+50 ± 10	-5 ± 9	+55 ± 10	-5 ± 5
50	-6 ± 11	-25 ± 14	+27 ± 7	-10 ± 6	+55 ± 10	-7 ± 5

[a]Time course after addition of cocaine.

[b]Determined as: Observed rate - Initial rate. Initial rate was measured after 90 minutes of tissue equilibration at which time cocaine was added.

[c]Number of observations.

0.36X; $r = 0.47$; $P < 0.05$; $n = 18$). Thus the lesser the rate the greater the response to cocaine.

Increasing the concentration of cocaine to 1×10^{-4} M depressed atrial rates at both 37° C and 26° C. In addition, in the presence of tropolone 1×10^{-4} M, cocaine consistently produced atrial irregularities (extrasystoles and asystoles).

DISCUSSION

Increased sensitivity to catecholamines has been observed in a variety of tissues when the bath temperature is decreased (see introduction). The present results, obtained by measuring the atrial chronotropic response to adrenergic amines, are in agreement with those observations, since cooling atria of mouse, rabbit and rat to 26° C resulted in increased potencies of these agonists when compared to potencies obtained at 37° C. The pD_2 value is an estimate of the affinity of an agonist for the receptor, and is an indication of the sensitivity of a tissue to a given agonist (Ariëns and Van Rossum, 1957).

Although quantitative differences were observed, the data show that TDS occurs in atria from all species examined. There are several possible explanations for TDS. A reduction of uptake of catecholamines into the adrenergic nerve terminals by low temperature could account for the TDS. However, in mouse atria cocaine did not prevent the potentiation of norepinephrine and epinephrine produced by a decrease in bath temperature. This is in agreement with results obtained from guinea-pig atria by Trendelenburg (1968) and Oppermann et al. (1971). Thus, blockade of neuronal uptake by low temperature does not seem to be the major mechanism for increased sensitivity to epinephrine and norepinephrine. Further support for this conclusion is given by results obtained with isoproterenol. The potency of this agonist was increased to a

greater extent than epinephrine and norepinephrine at 26° C in each species. Unlike norepinephrine and epinephrine, isoproterenol is not accumulated by adrenergic nerve terminals (Iversen, 1967).

Decreased activity of MAO and COMT at low temperature could also increase the potency of substrate agonists. That MAO is not involved in TDS is demonstrated by experiments by Oppermann et al. (1972), who found that inhibition of MAO by pargyline did not alter TDS. In addition it has been demonstrated that the activity of MAO is relatively unaltered at 30° C and 26° C (Schneider and Gillis, 1966; Oppermann et al., 1972). Furthermore, the present experiments demonstrate that TDS also occurs with isoproterenol, an agent not degraded by MAO (Hertting, 1964). The present results substantiate reports by other investigators (see introduction).

Since isoproterenol is catabolized by COMT, reduced activity of this enzyme at 26° C would be expected to result in increased tissue sensitivity to this and other agonists which are substrates for the enzyme. Accordingly, it was observed that the potencies of norepinephrine, epinephrine and isoproterenol increased at 26° C in the presence of cocaine. Conversely, the potencies of soterenol and nylidrin, agonists not degraded by COMT, were similar at 26° C and 37° C.

The hypothesis that COMT is involved in TDS was tested with the use of tropolone to block this enzyme. Since tropolone abolished TDS, decreased activity of COMT at 26° C may

be invoked as a possible mechanism for increased sensitivity to catecholamines at low temperature.

Decreased activity of COMT at 26° C may also be responsible for the positive chronotropic effect of cocaine. Since cocaine is not a direct-acting adrenergic receptor agonist and its effect was blocked by sotalol, it is likely that the atrial response was due to the release of endogenous norepinephrine. Trendelenburg (1968) demonstrated that reserpine pretreatment abolished the positive chronotropic effect of cocaine observed in guinea-pig atria at 37° C. Our experiments have demonstrated that at 26° C there is presumably a decreased activity of COMT; and, when combined with release of endogenous norepinephrine, this may result in increased concentrations of neurotransmitter at receptor sites. At 37° C the positive chronotropic effect of cocaine would not be observed since COMT is fully active under these conditions. This hypothesis is supported by the observation that inhibition of COMT by tropolone unmasked the atrial response to cocaine at 37° C. Even though cocaine was capable of producing a positive chronotropic response, it did not alter the potency of isoproterenol under any experimental conditions. This substantiates results of other investigators (Furchgott, 1967; Kimura et al., 1970; Westfall et al., 1972).

There is some indication that COMT is localized intracellularly and that catecholamines must gain access to the enzymatic sites in order to be O-methylated. In perfused rat hearts phenoxybenzamine blocks O-methylation of adrenergic

amines. However, when the tissue is homogenized, phenoxybenzamine no longer inhibits the O-methylation process (Eisenfeld et al., 1967). Phenoxybenzamine is believed to act by preventing extraneuronal uptake of catecholamines (Iversen, 1967). In addition it has been shown that phenoxybenzamine potentiates the effects of isoproterenol in several tissues (Foster, 1966; 1967; Stafford, 1963; Adler-Graschinsky et al., 1972; Kaumann, 1972). Moreover, it has been demonstrated that extraneuronal uptake is depressed by low temperatures (Gillespie et al., 1970). Therefore, diminished extraneuronal accumulation could be the factor involved in TDS. Different degrees of potentiation of catecholamines by blockade of extraneuronal uptake would result since these amines have different affinities for this uptake process. Since isoproterenol has greater affinity for extraneuronal uptake than does norepinephrine (Iversen, 1967), if at 26° C the extraneuronal accumulation is diminished, one would expect to see greater degree of potentiation of the effects of isoproterenol. The present data support this concept, since effects of isoproterenol were potentiated to a greater extent than those of norepinephrine. However, the results could also be interpreted as an indication that isoproterenol is a better substrate for COMT than is norepinephrine. Hence, reducing the activity of the enzyme by low temperature would also result in greater potentiation of isoproterenol. Giles and Miller (1967) demonstrated that the rate of O-methylation by rat liver COMT was faster for isoproterenol than for

norepinephrine.

One of the interesting findings of this study is the effect of low temperature and tropolone on effects of beta receptor antagonists. Slopes of pA_2 plots as well as pA_2 values were increased by both treatments. Differences in potency of different antagonists and low slope values of pA_2 plots of beta receptor antagonist have been reported in different tissues and with different agonists. In guinea-pig atria and trachea and in kitten atria beta blockers are less potent in antagonizing the effects of norepinephrine than those of isoproterenol (Foster, 1966; Otorii, 1969; Chahl and O'Donnell, 1967; Moore and O'Donnell, 1970; Blinks, 1967; Kaumann, 1970). It has been suggested that since norepinephrine is removed by adrenergic nerve terminals, the antagonist might reduce the uptake process, thus concealing the degree of antagonism experimentally observed (Foster, 1966; Blinks, 1967; Kaumann, 1970). An alternative explanation is that neuronal uptake might become saturated by the high doses of agonists (Chahl and O'Donnell, 1967; Blinks, 1967; Langer and Trendelenburg, 1969). Using isoproterenol as the agonist, low slope values of pA_2 plots of beta blockers have also been observed by different investigators (Patil, 1968; Moore and O'Donnell, 1970; Buckner and Patil, 1971; Otorii, 1969; Levy and Wilkenfeld, 1970; Bristow et al., 1970; Levy and Wasserman, 1970; Wasserman and Levy, 1972). It is assumed that the reduced slope is not related to the neuronal uptake process, since isoproterenol is not taken up intraneuronally

(Iversen, 1967). The present results substantiates those observations since propranolol yielded slope values lower than the expected value of 1.

Slope values of pA_2 plots lower than the theoretical value of 1 for competitive beta receptor antagonists have been explained in terms of binding of the antagonist to sites other than the receptors (Bristow et al., 1970; Levy and Wilkenfeld, 1970). In trachea, a tissue that must be contracted in order to analyze the relaxing effect of catecholamines, it was postulated that low slope values could be due to a non-competitive interaction between the blocker and the spasmogen (Patil, 1968). A relaxing effect of the antagonist on the same tissue has also been cited as the reason for low slope values (Moore and O'Donnell, 1970). After observing that blockade by propranolol of the lipolysis produced by catecholamines, in rats, was not proportional to the increase in antagonist concentration, Wenke et al. (1967) postulated that combination of agonist and antagonist with receptors can be described by a quadratic type of interaction. According to their model a trimolecular reaction occurs (one molecule of agonist with two receptors or vice versa) rather than the usual bimolecular interaction (one molecule of agonist with one receptor). In this situation when the concentration of an antagonist, which interacts in a bimolecular way with the receptor, is increased 10-fold, the shift produced would be expected to be less than 1 log unit. The same model might be used to describe the antagonism by propranolol

of the glycogenolysis produced by catecholamines in rabbit liver slices. In this preparation 10-fold increases in antagonist concentration did not proportionally increase the dose ratio (Mühlbachova et al., 1972). Following the same reasoning Levy and Wilkenfeld (1970) attempted to explain low slope values for propranolol, pronethalol, practalol and sotalol on the basis of a multimolecular interaction between agonist and antagonist with the receptor. Moreover, based on differences in slope values of beta blockers in different tissues and species, it was postulated that the receptors involved were different (Levy and Wasserman, 1970; Wasserman and Levy, 1972).

The increase in the slope observed at 26° C in the case of propranolol indicate that factors other than the ones mentioned above may be involved. This factor(s) appears to be related to COMT, since prior inhibition of the enzyme by tropolone abolishes the effect of temperature. Furthermore, in the presence of tropolone slope values were not different from the theoretical value of 1. These results substantiate the observations by Buckner et al. (1971) and by Furchgott (1973) who observed that blocking the enzymatic process in guinea-pig trachea increased the slope of pA_2 plots of beta blockers. Two possibilities similar to those advanced in guinea-pig trachea (Buckner et al., 1971) may explain the low slope values of pA_2 plots of beta adrenergic antagonist: a) Saturation of the enzyme or access to the enzyme by high concentration of agonist at large dose ratios and b)

Inhibition of either process by the antagonist. That a saturable uptake and/or enzymatic process is present in mouse atria appears to be indicated by the changes observed in the slope of dose-response curves of catecholamines at low temperature and after treatment with tropolone. Under these conditions dose-response curves were flatter. The presence of a saturable process could lead to relatively steeper curves for agents subjected to such process (Langer and Trendelenburg, 1969). Inhibition of extraneuronal accumulation of catecholamine by dichloroisoproterenol has been observed in rat heart (Eisenfeld et al., 1967). It is possible that propranolol may have a similar effect in mouse atria.

If the greater potency of propranolol and sotalol at low temperature is related to decreased activity of COMT and/or extraneuronal uptake, then dose ratios using an agonist not degraded by COMT should be the same at either temperature. Furthermore, dose ratios should be the same as those obtained using isoproterenol as the agonist at 26° C. This hypothesis was tested with nylidrin. This agent is not a substrate for COMT. As predicted, the dose ratios for sotalol were the same at 37° C and 26° C. However, they were not as great as that obtained with isoproterenol-sotalol at 26° C. One possibility for this discrepancy is that nylidrin may have some extra beta receptor activating action. That nylidrin might act at sites other than beta receptors was suggested by Manley and Lawson (1968).

The increased potency of catecholamines observed at low temperature could be due to changes in beta receptors. Furthermore, the relative potency of isoproterenol to norepinephrine changed from 1.32:1 at 37° C to 3:1 at 26° C. Furchgott (1972) suggested that a change greater than 3-fold in the relative potencies of agonists and/or a change greater than 0.5 log units in the pA_2 values of antagonists may be used to suggest changes in receptor conformations. However, Kohli (1969) has suggested that relatively large differences in pA_x values, as much as 1 log unit, may occur between tissues without indicating differences in their receptors. The increase in the relative potency of isoproterenol to norepinephrine and the 5-fold increase in the pA_2 value of sotalol may indicate a change in receptors though not in the same direction hypothesized by others (Kunos and Szentivanyi, 1968; Buckley and Jordan, 1970; Broadley, 1972). Doubts about a change in receptors as a factor contributing to the increased potency of catecholamines at low temperature stem from results with propranolol and tropolone. Similar pA_2 values were obtained with propranolol at 37° C and 26° C in the presence of tropolone. It is then likely that the changes in pA_2 values for sotalol produced by low temperature could be abolished by tropolone. Changes in the order of potencies of catecholamines is not a precise way to investigate possible changes in receptors since agonists need not only combine with receptors but must trigger a response. However, assuming that there is a constant relationship between

intrinsic activity and number of receptors occupied by an agonist, then, under appropriate experimental conditions as outlined by Furchgott (1967; 1970; 1972), relative potencies of full agonist may be a useful criterion in investigations of receptor conformation.

Phentolamine shifted the dose-response curve of isoproterenol to the right, however, phenoxybenzamine an irreversible alpha adrenergic blocking agent had no effect on the potency of isoproterenol. This indicates that alpha adrenergic receptors are not involved in the chronotropic response to isoproterenol. The same conclusion was reached by Parr and Urquilla (1972) in rabbit atria and by Krell and Patil (1969) in guinea-pig atria. One interesting observation is that atropine displayed blocking activity to the chronotropic effect of isoproterenol at 26° C but not at 37° C. This blocking action of atropine appears to be non-competitive in nature, since increasing the dose of antagonist did not proportionally increase the dose ratio. The capacity of atropine to antagonize the epinephrine effects has been reported in a variety of tissues (Bussel, 1940). More recently Allan et al. (1969) suggested a possible alpha adrenergic blocking property of atropine to explain the decrease in blood pressure produced by amphetamine in rats in the presence of the muscarinic antagonist. In those instances where atropine is found to block the effects of norepinephrine non-specificity has been advocated as the cause of such blockade. A non-specific action of atropine

may also be responsible for the shift observed at 26° C.

SUMMARY AND CONCLUSIONS

Under similar experimental conditions isolated atria from rabbit, rat and mouse were more sensitive to the chronotropic effect of catecholamines at 26° C than at 37° C. Low temperature potentiated the chronotropic response to isoproterenol more than that to norepinephrine. The sensitivity of isolated atria to the chronotropic effect of nylidrin and (-)-soterenol, agonists which are not substrates for COMT, was similar at 37° C and 26° C.

Tropolone, 1×10^{-5} M, potentiated the response of (±)-isoproterenol at both 37° C and 26° C. However, in the presence of tropolone, 1×10^{-4} M, the potency of (-)-isoproterenol was similar at each temperature. It appears then that since low temperature increased the potency of agents which are substrates for COMT and did not change that of agonists which are not metabolized by this enzyme, decreased activity of COMT is a factor involved in TDS. Abolition of the effect of temperature by tropolone, a COMT inhibitor, supports this conclusion.

At 26° C a positive chronotropic effect of cocaine was unmasked. This effect of cocaine was abolished by (±)-sotalol suggesting that endogenous norepinephrine is involved in that effect. At 37° C tropolone, 1×10^{-5} M, also unmasked the positive chronotropic effect of cocaine. Thus, the effect of cocaine seems to be brought about by endogenous norepinephrine in the presence of reduced COMT activity and blocked

neuronal uptake.

At 37° C the relative potencies of catecholamines in the presence of cocaine obtained in mouse atria (chronotropic response) were: (±)-isoproterenol 1.32, (-)-norepinephrine 1, (-)-epinephrine 0.21. At 26° C, the relative potencies were: (±)-isoproterenol 2.82, (-)-norepinephrine 1, (-)-epinephrine 0.22. This data confirm the role of beta adrenergic receptors in the production of chronotropic response in mouse atria. In addition, it suggests that since the order of potencies of catecholamines were the same at each temperature, there is probably no change in receptor type at 26° C in mouse atria.

At 26° C the pA_2 values of beta adrenergic antagonist increased. In addition, slope values of pA_2 plots of (±)-propranolol increased. The increased potency of beta receptor antagonists lend support to the conclusion that beta receptors are not transformed into another type at 26° C. Prior inhibition of COMT by tropolone abolished the effect of temperature on the potency of (±)-propranolol suggesting that COMT also influences the potency of beta receptor antagonists. The low slope values of pA_2 plots of (±)-propranolol at 37° C or in the absence of tropolone may be the result of inhibition of COMT or access to the enzyme by high concentrations of agonist or inhibition of the enzymatic process by the antagonist.

APPENDIX

Table 10

Chemical structures of the beta adrenergic agonists used in this study

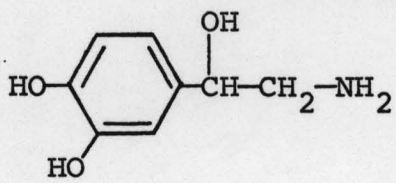
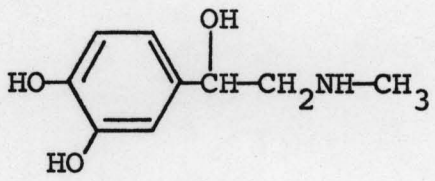
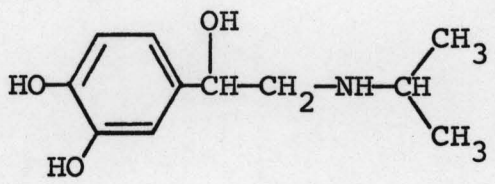
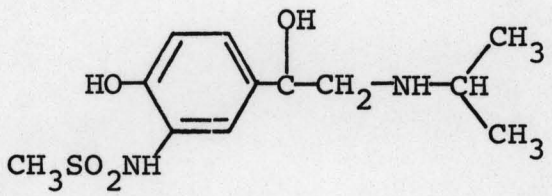
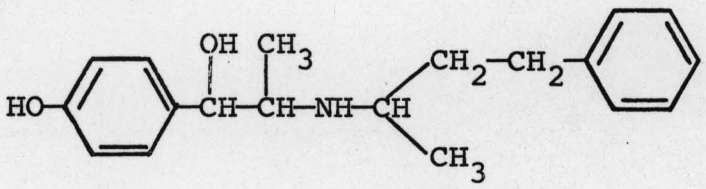
Compound	Structural formula
Norepinephrine	
Epinephrine	
Isoproterenol	
Soterenol	
Nylidrin	

Table 11

Influence of temperature on the potency of (±)-sotalol in mouse atria

Treatment[a]	37° C		26° C	
	(±)-Isoproterenol $pD_2 \pm$ S.E.M.	Dose ratio[b]	(±)-Isoproterenol $pD_2 \pm$ S.E.M.	Dose ratio[b]
None	6.79 ± 0.07 (8)		8.03 ± 0.11 (8)	
(±)-Sotalol, 1 x 10^{-5} M	6.05 ± 0.03 (8)	5	6.63 ± 0.11 (8)	25
(±)-Sotalol, 1 x 10^{-4} M	5.28 ± 0.03 (8)	32	5.75 ± 0.12 (8)	195

[a]Tissues were incubated with sotalol for 45 minutes.

[b]Dose ratio = Antilog (pD_2 without antagonist - pD_2 with antagonist).

Numbers in parentheses are number of observations.

Table 12

Influence of temperature and tropolone on the potency of (±)-propranolol in mouse atria

Treatment[a]	Without tropolone				With tropolone[b]			
	37° C		26° C		37° C		26° C	
	(±)-Isoproterenol $pD_2 \pm$ S.E.M.	Dose ratio[c]	(±)-Isoproterenol $pD_2 \pm$ S.E.M.	Dose ratio[c]	(±)-Isoproterenol $pD_2 \pm$ S.E.M.	Dose ratio[c]	(±)-Isoproterenol $pD_2 \pm$ S.E.M.	Dose ratio[c]
None	6.73 ± 0.08 (7)		7.76 ± 0.09 (6)		8.20 ± 0.06 (5)		8.85 ± 0.14 (6)	
(±)-Propranolol, 1×10^{-8} M	6.20 ± 0.03 (7)	3	6.86 ± 0.09 (7)	8	7.21 ± 0.11 (6)	9	7.63 ± 0.10 (6)	17
(±)-Propranolol, 1×10^{-7} M	5.77 ± 0.06 (7)	9	6.16 ± 0.06 (7)	39	6.52 ± 0.07 (6)	46	6.82 ± 0.11 (6)	109
(±)-Propranolol, 1×10^{-6} M	5.25 ± 0.05 (7)	30	5.51 ± 0.10 (7)	177	5.47 ± 0.04 (6)	518	5.70 ± 0.15 (7)	1430

[a]Tissues were incubated with propranolol for 45 minutes.

[b]Tropolone, 1×10^{-5} M was added 5 minutes prior to addition of the antagonist.

Table 12 (continued)

[c]Dose ratio = Antilog (pD_2 without antagonist - pD_2 with antagonist).

Numbers in parentheses are number of observations.

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ANALYSIS OF TEMPERATURE-DEPENDENT SENSITIVITY OF ISOLATED ATRIA TO ADRENERGIC AGONISTS AND ANTAGONISTS

BY HECTOR MUNOZ-RAMIREZ

Under the supervision of Assistant Professor Carl Kenneth Buckner and Associate Professor Charles F. Ryan

The influence of temperature on the sensitivity of isolated atria to catecholamines was examined at 37° C and 26° C. Atria from rabbit, rat and mouse were more sensitive to the chronotropic effect of (-)-isoproterenol at 26° C than at 37° C. Rabbit atria were also found to be more sensitive to the chronotropic effect of (±)-norepinephrine at 26° C. In the presence of cocaine mouse atria were more sensitive to the chronotropic effect of (±)-isoproterenol, (-)-norepinephrine, and (-)-epinephrine at 26° C than at 37° C. Low temperature potentiated the chronotropic effect of (±)-isoproterenol more than that of (-)-norepinephrine. The potency of (±)-isoproterenol in the presence of the catecholamine-O-methyltransferase (COMT) inhibitor tropolone, 1×10^{-5} M, was greater at 26° C. However, in the presence of tropolone, 1×10^{-4} M, the potency of (-)-isoproterenol was similar at 37° C and 26° C. The sensitivity of isolated atria to the chronotropic effect of nylidrin and (-)-soterenol, non-catechol beta adrenergic agonists were also examined. The potencies of these agents were not increased at 26° C.

The data suggest that decreased activity of COMT influences the sensitivity of isolated atria to catecholamines.

Cocaine, 1×10^{-5} M, produced a positive chronotropic effect at 26° C but did not do so at 37° C. The cocaine chronotropic effect was blocked by (±)-sotalol, 1×10^{-6} M, suggesting that the response to cocaine is mediated by endogeneous norepinephrine. At 37° C, tropolone, 1×10^{-5} M, unmasked the positive chronotropic effect of cocaine. Thus, the response to cocaine appears to be due to a combination of decreased activity of COMT and neuronal release of norepinephrine.

The effect of temperature on adrenergic receptors of mouse atria was studied using classical criteria. At 37° C the relative potencies for the chronotropic effect of catecholamines were: (±)-isoproterenol 1.32, (-)-norepinephrine 1, (-)-epinephrine 0.21. At 26° C the relative potencies were: (±)-isoproterenol 2.82, (-)-norepinephrine 1, (-)-epinephrine 0.22. These data indicate that beta adrenergic receptors mediate the chronotropic response to catecholamines in mouse atria at both 37° C and 26° C.

Different antagonists were also used to evaluate the role of receptors in the increased sensitivity of mouse atria to catecholamines. Atropine, significantly antagonized the chronotropic effect of (-)-isoproterenol in a non-specific manner at 26° C. However, atropine had no effect at 37° C. Phentolamine decreased the potency of (-)-isoproterenol at 37° C but not at 26° C. The non-competitive alpha receptor

antagonist phenoxybenzamine did not alter the potency of (±)-isoproterenol suggesting that alpha receptors do not mediate the isoproterenol chronotropic effect in mouse atria. Racemic sotalol displayed greater potency in antagonizing the chronotropic response to isoproterenol at 26° C than at 37° C. Plots of log (dose ratio - 1) vs negative log molar sotalol concentration yielded a pA_2 value of 5.79 at 37° C and 6.48 at 26° C. With nylidrin as the agonist the potency of sotalol was similar at 37° C and 26° C. Dose ratios obtained with nylidrin-sotalol were smaller than those obtained with isoproterenol-sotalol at 26° C. The reason for this discrepancy might be due to an action of nylidrin at sites other than beta receptors. The beta adrenergic antagonist (±)-propranolol yielded pA_2 values of 8.86 and 9.20 at 37° C and 26° C, respectively. With the same antagonist, regression coefficients of pA_2 plots were 0.54 at 37° C and 0.70 at 26° C. In the presence of tropolone, 1×10^{-5} M, pA_2 values of propranolol were 9.0 at 37° C and 9.17 at 26° C. Tropolone, 1×10^{-5} M, increased slope values of pA_2 plots of (±)-propranolol to values equal to 1 at 37° C and 26° C. A slope value of 1 is to be expected if the antagonist is acting competitively.

The data obtained in this study suggest that decreased activity of COMT has a definite influence on the sensitivity of isolated atria to catecholamines at low temperature. The data also indicate that since there is no change in the order of potencies of catecholamines, and the apparent potency of

beta receptor antagonists is actually increased, there is probably no change in receptor type at 26° C in mouse atria. The increase in the observed potency of competitive beta receptor antagonist and the slope values of pA_2 plots obtained at 26° C, seem to be the result of COMT inhibition. The data obtained with tropolone indicate that prior inhibition of COMT abolishes the effect of temperature on these parameters. The reasons for the low slope values of pA_2 plots at 37° C or in the absence of tropolone could be due to inhibition of COMT or access to the enzyme by high concentrations of the agonist at large dose ratios or inhibition of the enzymatic process by the antagonist. The present results do not allow any conclusion about which mechanism is operative.

APPROVED: Carl K. Buckner

Carl K. Buckner, Ph.D.
Assistant Professor

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