J. Physiol. (1982), **333**, *pp.* 511–529
With 7 text-figures
Printed in Great Britain

THE THERMOREGULATORY EFFECTS OF NORADRENALINE, SEROTONIN AND CARBACHOL INJECTED INTO THE RAT SPINAL SUBARACHNOID SPACE

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(*Received 29 September 1981*)

SUMMARY

1. We have examined the effects on thermoregulation in the rat of noradrenaline bitartrate (NA), 5-hydroxytryptamine hydrochloride (5-HT) and carbamylcholine chloride (CCh) injected into the lumbar spinal subarachnoid space via a chronic indwelling catheter.

2. Intrathecal injections of the monoamines and CCh reproducibly affected thermoregulation, whereas injections of control solutions had no effect.

3. Intrathecal injections of NA (0·01–0·30 μmol) produced a dose-dependent hypothermia associated with a decrease in tail skin vasomotor tone. Shivering activity was not depressed during the hypothermia and sometimes increased. Intrathecal administration of the α-adrenergic agonist clonidine (0·0175–0·070 μmol) elicited changes in T_c and T_{sk} similar to those induced by intrathecal NA.

4. Intrathecal 5-HT (0·030–0·90 μmol) elicited a dose-dependent hyperthermia accompanied by increased tail skin vasomotor tone and increased shivering.

5. CCh injected intrathecally (0·001–0·06 μmol) evoked a dose-dependent hyperthermia. During the period when core temperature was rising, tail skin vasomotor tone increased and shivering-like activity was present. Once the maximum core temperature had been reached, tail skin vasodilatation occurred. Vasodilatation persisted until core temperature had returned to normal.

6. Intravenous injections of 5-HT (0·30 and 0·90 μmol) or CCh (0·006 and 0·03 μmol) caused no thermoregulatory effect. The effects of these agents injected intrathecally were therefore not due to an action in the periphery.

7. Intravenous infusions of NA (0·06 and 0·10 μmol) produced hypothermia and transient tail skin vasodilatation. We suggest that an action at peripheral sites may have contributed to the effects produced by intrathecal injection of this monamine.

8. These findings suggest that spinal noradrenergic, serotonergic and cholinergic synapses may be importantly involved in the control of body temperature in the rat. The possible functional roles of these synapses and the putative spinal sites of action of the injected substances are discussed.

INTRODUCTION

Historically, the hypothalamus and the adjacent preoptic area have been considered as the 'seat of thermoregulation'. However, in recent years it has become clear that other regions of the central nervous system (C.N.S.) are importantly involved in thermoregulatory processes and, in fact, are capable of a limited degree of autonomous control (Lipton, 1973; Lin & Chai, 1974; Simon, 1974). One such C.N.S. region is the spinal cord. Of obvious importance is the spinal cord's role as an information conduit. The cord relays thermal information from peripheral thermodetectors to supraspinal structures and conveys signals from these structures to the various thermoregulatory effector systems. However, the cord in addition displays an integrative capacity. That is, afferent and efferent information is significantly modified at the spinal level before it is forwarded to the supraspinal centres and the peripheral effectors, respectively (Christensen & Perl, 1970; Hellon & Misra, 1973; Hilton & Spyer, 1980). The cord also contains a population of thermodetector neurones. Heating and cooling of the cord elicits co-ordinated thermoregulatory responses (Simon, 1974). Thus, the cord is importantly involved in temperature regulation and shares with the hypothalamus/preoptic region the attributes of thermosensitivity and integrative capacity.

The hypothalamus is heavily innervated by monoaminergic and cholinergic fibres (Shute & Lewis, 1966; Kent & Sladek, 1978), and the role of these hypothalamic neural systems in thermoregulation has been extensively investigated (Hellon, 1975). Monoaminergic and cholinergic pathways also innervate several areas of the spinal cord. Available evidence indicates that noradrenergic, serotonergic and cholinergic nerve terminals are located in the dorsal and ventral horns of the spinal cord grey matter of the rat (Carlsson, Falck, Fuxe & Hillarp, 1964; Kasa, 1975; Nygren & Olsen, 1977). In addition, terminals are found in the intermediolateral nucleus (i.m.l.) of the grey matter, which contains the cell bodies of the preganglionic sympathetic fibres (Kasa, 1975; Glazer & Ross, 1980; Loewy & McKeller, 1981). The possibility therefore exists that the spinal neurotransmitters, noradrenaline, 5-hydroxytryptamine and acetylcholine, like their hypothalamic counterparts, are involved in the control of body temperature. However, no investigations of their putative roles have been carried out. In the present study, we examined the effects on thermoregulation of noradrenaline, 5-hydroxytryptamine and carbamylcholine injected directly into the spinal subarachnoid space of the rat.

METHODS

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

Drugs

Drugs used were l-noradrenaline bitartrate (NA), 5-hydroxytryptamine hydrochloride (5-HT), carbamylcholine chloride (CCh) (Sigma Chemical Company, St. Louis, MO, U.S.A.) *d*-noradrenaline bitartrate (*d*-NA) (Adams Chemical Co.) and clonidine hydrochloride (Boehringer Inglheim Ltd., Ridgefield, CT, U.S.A.). Drugs were dissolved in an artifical cerebrospinal fluid (a.c.s.f.) described by Yeung & Rudy (1980). Drug solutions were prepared daily.

Surgical procedures

Catheterization of the spinal subarachnoid space. Rats were anaesthetized with pentobarbitone and prepared with chronic indwelling spinal catheters according to the method of LoPachin, Rudy & Yaksh (1981). The spinal catheters were formed from polyethylene tubing (PE 10; o.d. = 0·61 mm) and the section of each catheter which was inserted into the subarachnoid space was stretched so that the outside diameter was reduced by approximately 30%. The tips of the catheters were situated in the spinal subarachnoid space at the level of lumbar enlargement. Before implantation, the volume of each catheter was measured. Mean capacity was 7 μl (range = 6·7–7·4 μl). Rats were allowed to recover from surgery for at least 1 week before they were used in an experiment. At the conclusion of all experiments, the rats were killed with pentobarbitone, and the position of the spinal catheter within the subarachnoid space was determined.

Intravenous catheterization. Rats were prepared with intravenous catheters fashioned from polyethylene tubing (PE10). The right external jugular vein was exposed by a small incision in the neck. The catheter was inserted through the vein to the junction of the vena cava with the right atrium. The catheter was tied into the vein, and the free end of the tubing was brought subcutaneously to exit through a stab wound at the nape of the neck. The rats were allowed to recover from surgery for a week. Daily injections of heparinized saline maintained the patency of the catheter.

Injection techniques

Intrathecal injections of control and drug solutions were carried out using a 10 μl Hamilton syringe with a 30 gauge permanently affixed needle. The syringe was attached directly to the exterior portion of the spinal catheter and was driven by a hand-held micrometric syringe buret. An intrathecal injection was performed by loading the spinal catheter (which had a volume of 7 μl, *vide supra*) with 4 μl of drug or control solution. The catheter was then flushed with 8 μl of a.c.s.f. to ensure that the pre-loaded solution was expelled completely into the subarachnoid space. Intrathecal injections required approximately 2 min to complete.

As a control for the intrathecal injections of noradrenaline bitartrate, a solution of sodium bitartrate (Fisher Scientific Co., Fair Town, NJ, U.S.A.) was prepared in sterile a.c.s.f. The pH, osmolarity and bitartrate concentration of this solution were adjusted to match those of the highest dose of NA used in these experiments (0·30 μmol NA = 605 mosmol, pH = 3·7). The control solutions for the chloride ion, pH and osmolarity of the 5-HT, CCh and clonidine solutions were prepared by adding sufficient sodium chloride to a volume of a.c.s.f. to make the solution isotonic with respect to the highest dose of drug used (0·90 μmol 5-HT = 605 mosmol, 0·06 μmol CCh = 274 mosmol, 0·07 μmol clonidine = 290 mosmol). The final solution was brought to the appropriate pH with 0·05 N-HCl (5-HT = pH 2·77, CCh = pH 6·2, clonidine = pH 6·5).

The prepared solutions to be injected were sterilized by forcing them through a millipore filter unit (Millex®-GS, 0·22 μm filter unit). The solutions were received into sterilized test tubes and used within 30 min. The Hamilton glass microlitre syringes used for intrathecal and intravenous injections were stored in 95% alcohol.

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

Recording of physiological responses

Colonic temperature (T_c) and tail skin temperature (T_{sk}) were continuously monitored throughout each experimental session. During some sessions electromyographic (e.m.g.) activity was recorded as an index of shivering.

To measure T_c, a YSI 401 thermistor probe (Yellow Springs Instrument Co.) was inserted 6 cm into the colon. T_{sk} was assessed with a YSI 427 thermistor disk applied to the ventral surface of the tail approximately 2 cm from the anus. The tail and colonic thermistors were held in place by taping the leads to the rat's tail. T_c and T_{sk} were continuously recorded using a multichannel potentiometric recorder.

In some experiments, the e.m.g. activity of the lateral thigh muscles of one hind leg was recorded according to method of Ackerman & Rudy (1980). At the beginning of each experimental session in which e.m.g. activity was recorded, the rat was warmed with an infra-red light to suppress

shivering. The residual background activity was subtracted from the e.m.g. values recorded during the session. Intrathecal injections of 1-NA occasionally produced myoclonic contractions which contaminated the e.m.g. records (*vide infra*). A combination of electronic and manual adjustment of the data were used to subtract out artifacts arising from these myoclonic jerks.

E.m.g. data were acquired as both raw e.m.g. tracings and as integrated e.m.g. activity. The raw tracings were used to monitor the qualitative aspects of e.m.g. activity. Throughout the text 'shivering-like e.m.g. activity' refers to drug-induced changes in e.m.g. tracings which were qualitatively similar to e.m.g. tracings associated with cold-induced shivering. Integrated e.m.g. was averaged over 2 min periods and provided an estimate of the quantity of shivering-like activity per unit time. Integrated e.m.g. was reported in arbitrary units of millimetre pen rise per minute.

TABLE 1. Drugs and doses injected into the spinal subarachnoid space

	μmol	μg salt	μg base
1-Noradrenaline bitartrate	0·01	3·19	1·69
	0·03	9·58	5·08
	0·06	19·16	10·01
	0·10	31·93	16·92
	0·30	95·79	50·77
5-Hydroxytryptamine hydrochloride	0·03	6·38	5·29
	0·10	21·27	17·65
	0·30	63·81	52·96
	0·60	127·62	105·92
	0·90	191·43	158·88
Carbamylcholine chloride	0·001	0·18	0·15
	0·003	0·55	0·44
	0·006	1·10	0·89
	0·01	1·83	1·48
	0·03	5·47	4·42
	0·06	10·96	8·85
Clonidine hydrochloride	0·0175	4·00	3·40
	0·035	8·00	6·80
	0·070	16·00	13·60

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

Quantification of thermoregulatory responses

The ability of drugs injected intrathecally to produce changes in rat thermoregulation was expressed in terms of the following descriptive parameters. ΔT_c(°C): the maximum change in T_c produced by a drug or control treatment (ΔT_c^+ = maximum increase in T_c; ΔT_c^- = maximum decrease in T_c). The change was measured relative to the rat's baseline T_c. The thermal index (t.i.)(°C . h) was derived by planimetric measurement of the area between the curve representing the treatment-induced change in T_c or T_{sk} and the extrapolated base line. The rate of temperature change(r.t.c.)(°C/h) represents the average rate at which colonic temperature changed during the first 50% of the total drug effect on T_c (r.t.c.$^-$ = rate of temperature decrease; r.t.c.$^+$ = rate of temperature increase). This is the period during which the rate of T_c change is maximum. ΔT_{sk}(°C): the maximum change in T_{sk} produced by drug or control treatment (ΔT_{sk}^+ = maximum increase in T_{sk}, ΔT_{sk}^- = maximum decrease in T_{sk}). The change was measured relative to the rat's base line T_{sk}.

Determination of dose–response characteristics

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

Rats were prepared with spinal catheters and divided into four groups of six to eight animals each. Each group received one of the drugs listed in Table 1. The rats of a particular drug group were started on a crossover design in which individual animals received intrathecal injections of a control solution and of each of the different doses of the designated drug. The group of rats which received l-noradrenaline also received a single injection of *d*-noradrenaline (0·30 μmol). The control solution and doses of drug were administered according to a randomized schedule. Intrathecal injections in a given rat were separated by at least two days. The data for each dose–response curve were analysed by a two-way (treatments × rats) analysis of variance (Winer, 1962), and the means for each dose of drug were compared at the 0·05 level of significance using Duncan's multiple range test (Freund, Livermore & Miller, 1960).

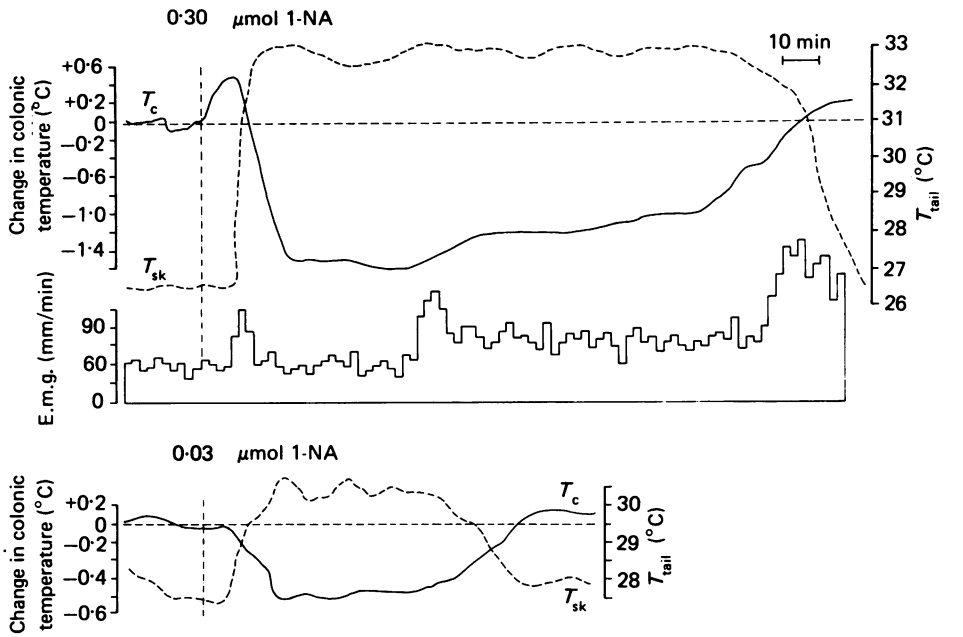

Fig. 1. The upper panel is an example obtained from one rat of the effects on colonic temperature (T_c), tail skin temperature (T_{sk}), and electromyographic activity (e.m.g.) produced by intrathecal injection of the 0.30 μmol dose of NA. The lower panel shows the effect in the same rat of an intrathecal injection of 0·03 μmol of NA. E.m.g. activity was not recorded in this experiment. Vertical dashed lines correspond to the time of NA injection.

RESULTS

General characteristics of the effects of intrathecal adrenergic agonists on thermoregulation

The lowest dose of NA tested (0·01 μmol) produced no consistent thermoregulatory effect. The major effect of the other doses (0·03–0·30 μmol) was the production of hypothermia associated with an increase in T_{sk} (Fig. 1). With the 0·03–0·10 μmol doses, T_c began to fall approximately 5–7 min after intrathecal injection. At the highest dose (0·30 μmol), the hypothermia was preceded by a small, transient rise in T_c (0·41 ± 0·13 °C). Thus, at this dose, the start of the hypothermic response was

delayed for the duration of the initial hyperthermia (about 20–25 min). In fourteen sessions (58 %), T_{sk} began to rise and T_c began to fall at the same time. During these sessions, the return of T_c and T_{sk} toward normal occurred concomitantly. However, in ten sessions (42 %), the hypothermia was well developed before the change in T_{sk} began. In these experiments, a similar lack of correlation between T_c and T_{sk} was observed during the return of T_c to base line, i.e. T_c returned to normal in advance of T_{sk}.

In a separate group of rats, the effects of intrathecal injections of two doses of NA on e.m.g. activity were examined. During these experiments, T_c and T_{sk} were also recorded. Intrathecal administration of control solutions did not alter e.m.g. activity. In four rats given intrathecal injections of 0·10 and 0·30 μmol NA, changes in e.m.g. activity did not occur during the hypothermic response until T_c began to return toward normal. The return of T_c to base line was associated with an increase in shivering-like e.m.g. activity. However, in two other rats given the 0·30 μmol dose of NA, shivering-like activity increased during the fall in T_c and remained above the pre-injection level until T_c returned to base line. It is noteworthy that the initial hyperthermic response produced by the 0·30 μmol dose was not associated with an increase in shivering-like e.m.g. activity, and that a depression of e.m.g. activity did not occur during the fall in T_c produced by either of the doses tested.

Intrathecal injections of NA control solutions did not alter rat thermoregulation. Moreover, injections of the *d*-isomer of NA (0·30 μmol) did not produce significant changes in T_c or T_{sk} in four rats. However, in two rats, intrathecal *d*-NA produced a small, short lasting fall in T_c associated with a rise in T_{sk}.

Intrathecal injections of NA elicited several non-thermoregulatory effects. Myoclonic muscle contractions were observed in some rats at all doses tested. The myoclonic contractions affected the hind limbs and resembled stepping movements. They occurred about 5–8 min after NA injection and lasted less than 5 min. These muscle contractions were more prevalent at the 0·30 μmol dose of NA. However, they probably did not contribute to the hyperthermia associated with this dose since myoclonus was also seen at doses which did not cause hyperthermia. Athetoid tail movements were also observed after intrathecal injections of NA at all dose levels. Their onset was similar to the myoclonic contractions, but they lasted longer (about 15–30 min).

Injection of clonidine (see Table 1 for doses) into the spinal subarachnoid space produced an effect on thermoregulation similar to that produced by intrathecal administration of the 0·03–0·10 μmol dose of NA, i.e. a monophasic hypothermia associated with a prolonged increase in T_{sk}. Intrathecal injections of clonidine control solutions did not alter T_c or T_{sk}. Moreover, non-thermoregulatory changes in behaviour or motor function were not observed following intrathecal clonidine.

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

The previous section indicates that a fall in T_c in conjunction with a rise in T_{sk} were the major effects of intrathecal NA and clonidine on rat thermoregulation. For each agonist, statistical analysis indicated that ΔT_c^- (Fig. 2*A*) r.t.c. (Fig. 2*B*) and the t.i. of the hypothermia (Fig. 2*C*) were all dose-dependent. Although both NA and

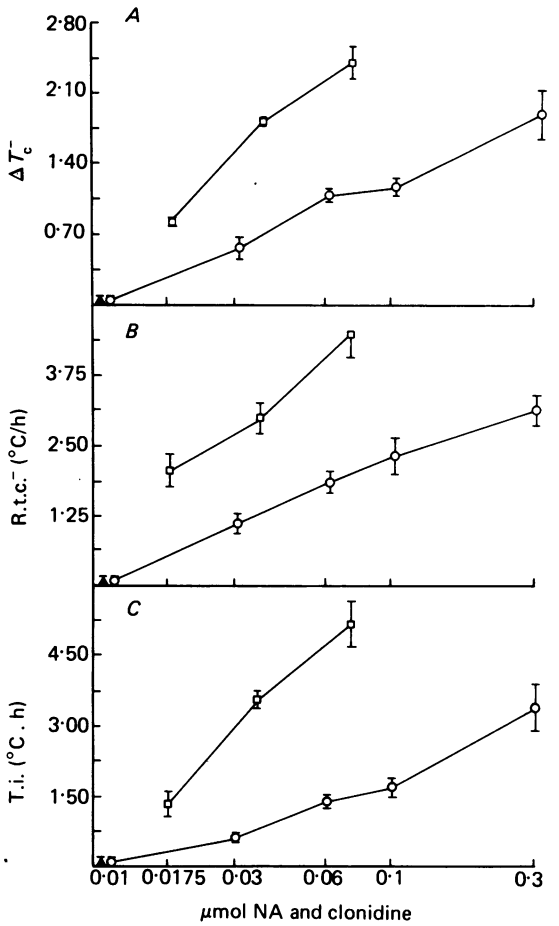

Fig. 2. Dose–response relationships for the effects of NA (○) and clonidine (□) on the maximum fall in colonic temperature (ΔT_c^-, *A*), the rate of temperature decrease (r.t.c.$^-$, *B*), and the thermal index (t.i., *C*). Each datum point represents a mean based on observations in six to eight rats. Vertical bracket lines indicate S.E. of the mean. The filled triangle in the lower left corner of each panel denotes the response to injections of a control solution.

clonidine produced a trend toward a larger ΔT_{sk}^+ with increasing dose, this relationship for either agonist was not statistically significant (data not shown). The ΔT_{sk}^+ for both agonists ranged from 5 to 7 °C. However, the duration, and thus the t.i., of NA- and clonidine-induced tail skin vasodilatation was found to be dose-dependent (data not shown).

General characteristics of the effects of intrathecal 5-HT on thermoregulation

Intrathecal injection of 5-HT caused an immediate, rapidly developing increase in T_c (Fig. 3, see Table 1 for doses). During the rising phase of the hyperthermia, T_{sk} either decreased slightly or remained unchanged. With the smaller doses of 5-HT (0·03–0·10 μmol), T_c rose rapidly to a maximum and then immediately began to

return toward base line, whereas with the higher doses (0·30–0·90 μmol), T_c entered a plateau phase before returning to normal. T_c and T_{sk} recovered concurrently in those rats in which intrathecal injection of 5-HT induced an initial tail skin vasoconstriction.

Changes in e.m.g. activity induced by intrathecal injections of 0·30 and 0·60 μmol 5-HT were assessed in five rats. Both doses of 5-HT produced marked increases in shivering-like e.m.g. activity which were temporally related to the rising T_c.

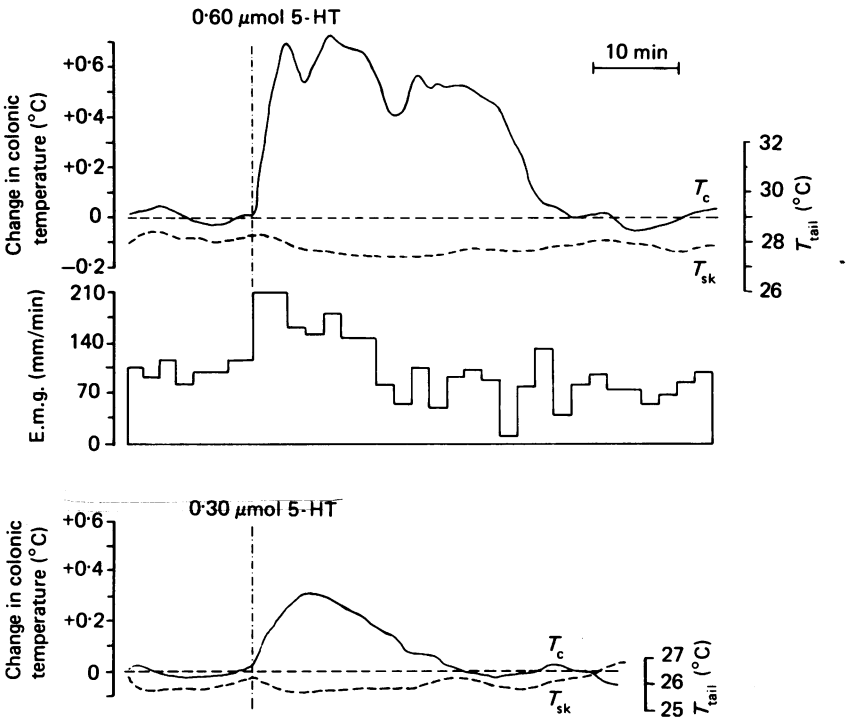

Fig. 3. The upper panel shows an example obtained from one rat of the effects on colonic temperature (T_c), tail skin temperature (T_{sk}), and electromyographic activity (e.m.g) produced by intrathecal injection of the 0·60 μmol dose of 5-HT. The lower panel shows the effect in the same rat of an intrathecal injection of 0·30 μmol of 5-HT. E.m.g. activity was not recorded in this experiment. Vertical dashed lines correspond to the time of 5-HT injection.

Moreover, the changes in e.m.g. recordings were accompanied by intense shivering-like activity which was visually evident in the hind quarters of the animal. For the 0·30 μmol dose, once the maximum T_c was attained, e.m.g. activity returned to pre-injection levels or below. In contrast, at the 0·60 μmol dose, e.m.g. activity remained elevated through at least the first portion of the plateau phase of the T_c response and then fell to pre-injection levels or below.

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

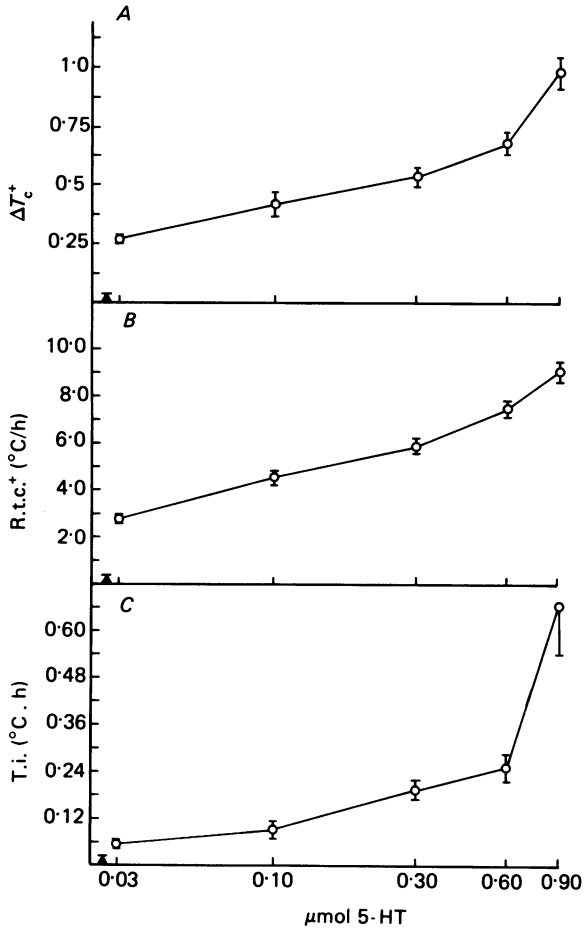

Fig. 4. Dose–response relationships for the effects of 5-HT on the maximum increase in colonic temperature (ΔT_c^+, *A*), the rate of temperature increase (r.t.c.$^+$ *B*), and the thermal index (t.i., *C*). Each datum point represents a mean based on observations in six to eight rats. Vertical bracket lines indicate S.E. of the mean. The filled triangle in the lower left corner of each panel denotes the response to injections of a control solution.

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

Intrathecal 5-HT injections produced dose-dependent changes in ΔT_c^+ (Fig. 4*A*), r.t.c.$^+$ (Fig. 4*B*) and the t.i. of the hypenthermia (Fig. 4*C*).

General characteristics of the effects of intrathecal CCh on thermoregulation

Intrathecal administration of CCh produced an immediate, rapidly developing monophasic hyperthermia (Fig. 5; see Table 1 for doses). During the rising phase of the T_c response, T_{sk} fell slightly or remained constant. However, T_{sk} began to increase once the maximum T_c was attained. With the 0·01–0·06 μmol doses of CCh, T_c was maintained at a plateau. During this plateau phase, T_{sk} reached a maximum, and shortly thereafter T_c began to return to normal. Generally, T_{sk} remained elevated

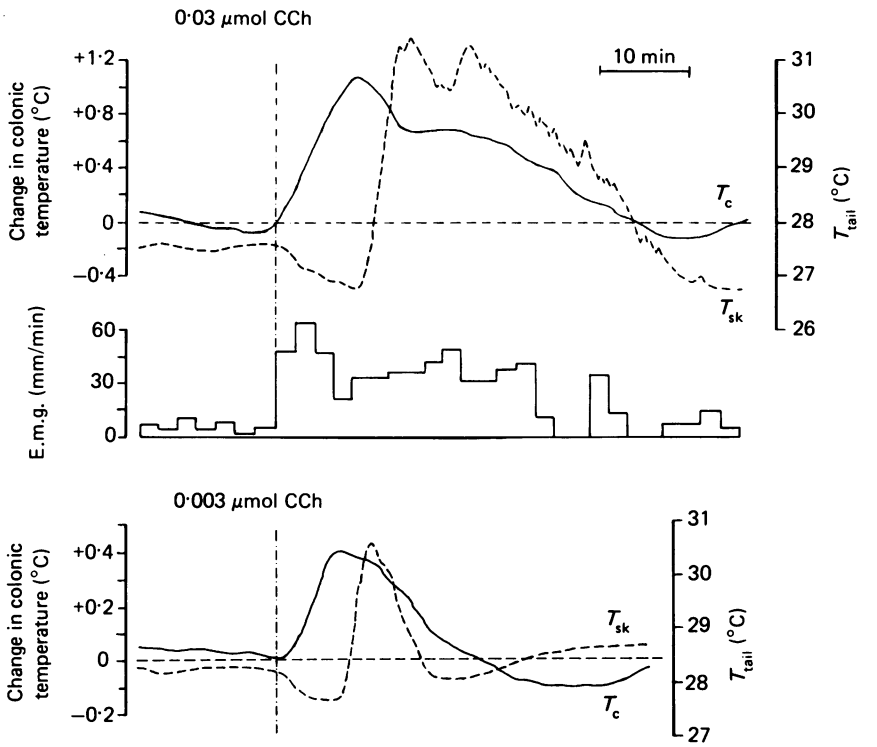

Fig. 5. The upper panel shows an example obtained from one rat of the effects on colonic temperature (T_c), tail skin temperature (T_{sk}), and electromyographic activity (e.m.g.) produced by intrathecal injection of the 0·03 μmol dose of CCh. The lower panel shows the effect in the same rat of an intrathecal injection of 0·003 μmol of CCh. E.m.g. activity was not recorded in this experiment. Vertical dashed lines correspond to the time of CCh injection.

until T_c was at or near pre-injection temperature. The smaller doses of CCh (0·003–0·006 μmol) produced changes in T_c and T_{sk} which were qualitatively similar to the changes elicited by the higher doses. However, at these smaller doses, T_c and T_{sk} usually did not remain elevated but, rather, began to return to normal immediately after reaching a maximum.

The effects of two intrathecal doses of CCh (0·006 and 0·03 μmol) on e.m.g. activity were examined in four rats. Both doses of CCh caused an immediate increase in shivering-like e.m.g. activity. The 0·006 μmol dose of CCh induced a brief rise in e.m.g. activity which was associated with the initial portion of the rising T_c. E.m.g. activity then returned quickly to pre-injection levels or below. The larger dose of CCh (0·03 μmol) produced an increase in e.m.g. activity which remained elevated during the plateau phase of the T_c response. During the return of T_c toward normal, e.m.g. activity fell to pre-injection levels or below.

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

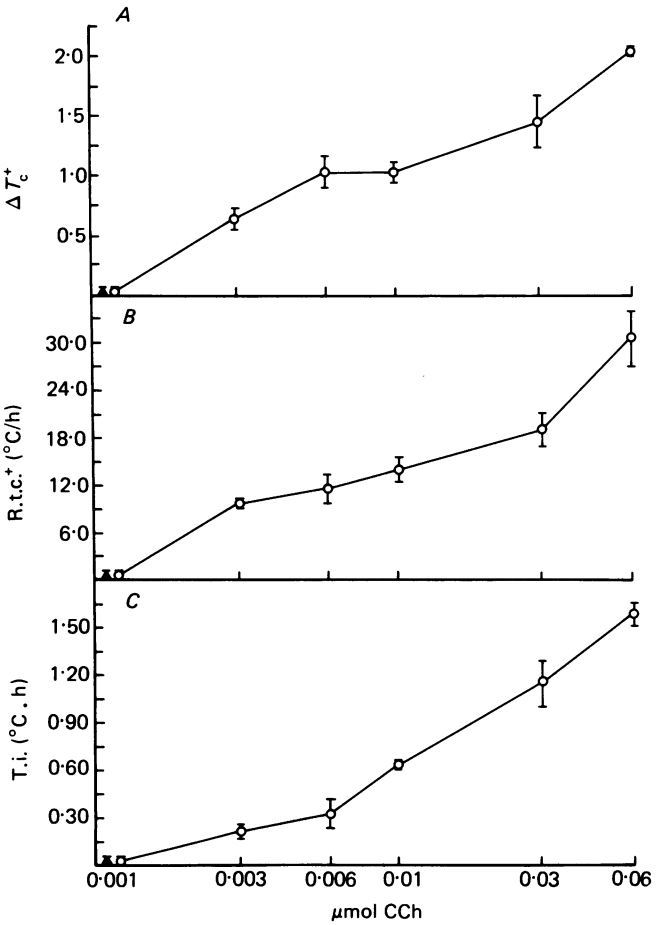

Fig. 6. Dose–response relationships for the effects of CCh on the maximum increase in colonic temperature (ΔT_c^+, *A*), the rate of temperature increase (r.t.c.$^+$, *B*), and the thermal index (t.i., *C*). Each datum point represents a mean based on observations in six to eight rats. Vertical bracketed lines indicate S.E. of the mean. The filled triangle in the lower left corner of each panel denotes the response to injections of a control solution.

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

Intrathecal injections of CCh promoted dose-related changes in ΔT_c^+ (Fig. 6*A*), r.t.c.$^+$ (Fig. 6*B*) and the t.i. of the hyperthermia (Fig. 6*C*).

As indicated above, intrathecal CCh caused a delayed increase in T_{sk}. The ΔT_{sk}^+ produced by these injections was dose-dependent, with mean increases in T_{sk} ranging from 1·33 °C (0·003 μmol) to 5·92 °C (0·06 μmol) (data not shown). The t.i. of the CCh-induced tail skin vasodilation was also dose-dependent (data not shown).

Effects of intravenous administration of NA, 5-HT and CCh on thermoregulation

The possibility exists that some of the thermoregulatory effects of NA, 5-HT and CCh injected intrathecally could be due to a direct action on peripheral thermoeffectors following the leakage of these agents from their subarachnoid site of injection. To

test this possibility, we determined the effects on thermoregulation of intravenous (I.V.) administration of two doses of NA, 5-HT, and CCh. The doses of drug given I.V. were comparable to those given intrathecally. Moreover, with the exception of one of the NA doses, drugs given I.V. were infused over 5, 10 and 15 min periods to simulate several possible leakage rates of these agents from the spinal subarachnoid space.

No changes in thermoregulation were observed when 5-HT (0·30 and 0·90 μmol) and CCh (0·006 and 0·03 μmol were infused I.V. over 5, 10 and 15 min periods ($n = 6$).

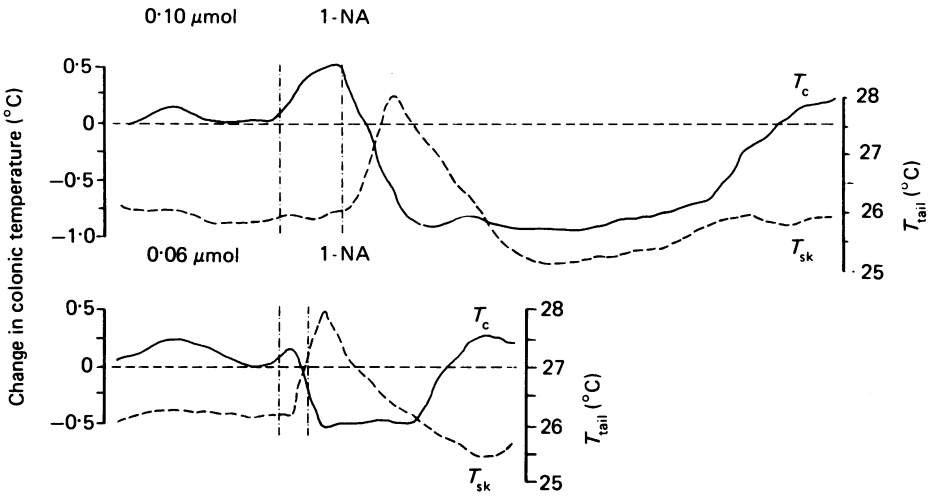

Fig. 7. Examples of the effects on colonic temperature (T_c) and tail skin temperature (T_{sk}) of intravenous infusions of NA. The 0·10 μmol and the 0·06 μmol doses of NA were infused over 10 and 5 min periods, respectively. Infusion periods are indicated by the vertical dashed lines. The data shown in the upper and lower panels were obtained from different rats.

In contrast, when NA was infused I.V., significant thermoregulatory responses were sometimes observed (Fig. 7). Infusion of 0·10 μmol of NA over a 10 min period produced hypothermia in four of six rats. Similar infusions of this dose of NA over 5 and 15 min periods caused hypothermia in three of six rats and two of six rats, respectively. Regardless of the infusion rate, when hypothermia occurred following infusion of the 0·10 μmol dose, T_c fell 0·80 °C ($\pm$0·10 °C). The hypothermic responses were usually preceded by a small rise in T_c which began during the infusion period. The changes in T_c were associated with a transient increase in T_{sk} which began during the 10 and 15 min infusion periods and immediately after the 5 min infusions of NA. Control solutions injected intravenously did not affect core or tail skin temperature.

I.V. infusion of 0·06 μmol of NA over a 5 min period caused a brief hypothermia in four of six rats which was not preceded by hyperthermia. The average fall in T_c was 0·45$\pm$0·05 °C for those rats in which hypothermia developed. The fall was associated with a transient increase in T_{sk}, which began during the course of the infusion.

DISCUSSION

The present study is the first investigation of the effects on thermoregulation of aminergic and cholinergic agonists injected into the spinal subarachnoid space. The main findings are that NA, 5-HT and CCh so injected produced clear-cut, dose-dependent changes in T_c and in the level of activity of certain thermoregulatory effectors. These findings are compatible with a possible role of spinal noradrenergic, serotonergic and cholinergic synapses in the control of body temperature.

Effects on thermoregulatory function of intrathecal NA, 5-HT and CCh

In theory, an agent injected into spinal subarachnoid space could alter body temperature by acting on either the afferent or the efferent aspect of the thermoregulatory system. An action on spinally situated thermodetectors or upon the rostrad transmission of impulses from thermodetectors in the skin of body core could introduce a false or biased input to the system. The functional consequence of a chemically mediated biasing of thermal afferent pathways would be to change the apparent level of the set point for thermoregulation. An agent which altered body temperature through an action on the efferent side of the system could do so by directly activating or inhibiting spinal pathways controlling peripheral thermoeffectors or by modulating the level of endogenous activity in these pathways. The result, in contrast to an effect on afferent systems, would be a relatively inflexible and possibly unco-ordinated pattern of changes in effector activities.

In the present experiments, injection of NA (0·30–0·30 μmol) and clonidine (0·0175–0·070 μmol) into the rat spinal subarachnoid space produced a dose-dependent fall in T_c and a rise in T_{sk}. The hypothermia evoked by the highest dose of NA (0·30 μmol) was preceded by a short hyperthermia. In a significant number of sessions (ten of twenty-four) the fall in T_c induced by NA began well in advance of the tail temperature response. Moreover, the hypothermia was not associated with an inhibition of shivering or thermoregulatory muscle tonus, since e.m.g. activity remained at the base line level or increased during the fall in T_c. This lack of co-ordination among thermoeffectors is not consistent with a NA-induced decrease in the set point level. Instead, we suggest that the effects of NA may have been the result of an action upon individual sympathetic effector systems such as those involved in the control of vasomotor tone and of non-shivering thermogenesis (n.s.t.) (*vide infra*).

Intrathecal injection of 5-HT caused an immediate, rapidly developing hyperthermia associated with co-ordinated changes in shivering and tail vasomotor tone. The concerted change in thermoeffector activities suggests that the hyperthermic response represents a 5-HT-induced increase in the set point mediated by an action within thermal afferent pathways. However, it must be stressed that simple observation of the pattern of effector activities at one ambient temperature, as was the situation in our experiments, is insufficient to differentiate a co-ordinated change in effector activities due to an action on afferent systems from a co-ordinated change resulting from the simultaneous, but independent, activation of several effector outflow pathways.

The effects of intrathecal CCh on thermoregulation are complex. CCh produced an

immediate, rapidly developing hyperthermia. As T_c rose, shivering-like activity increased and T_{sk} decreased. Once the maximum change in T_c had been reached, T_{sk} increased rapidly. At the 0·01–0·06 μmol doses of intrathecal CCh, the plateau phase of the T_c response was associated with continued shivering-like activity and tail skin vasodilatation. The thermoeffector co-ordination observed while T_c was rising suggests that the CCh response may have been mediated by an action on afferent pathways. However, the simultaneous presence of shivering and vasodilatation during the plateau phase suggests that CCh concomitantly activated thermogenic and heat dissipation mechanisms or that the vasodilatation was compensatory.

Receptor specificity of the effects of intrathecal NA, 5-HT and CCh

Several lines of evidence suggest that intrathecaly injected NA, 5-HT and CCh produced thermoregulatory effects by acting directly on agonist-specific pre- or post-junctional receptors. (1) The effects were not due to artifacts related to the volume of injection or the pH, osmolality or ionic content of the injectates, because injections controlling for these factors had no effect on thermoregulation. (2) Agonist-specific receptors with which the injected agents could have interacted are present in the spinal cord. Serotonergic receptors (Nelson, Herbet, Adrian, Bockaert & Hamon, 1980), α- and β- adrenergic receptors (Alexander, Davis & Lefkowitz, 1975; Young & Kuhar, 1979), and muscarinic and nicotinic cholinergic receptors (Myslinski & Randic, 1977; Kayaalp & Neff, 1980) have been found in several regions of the spinal cord grey matter. (3) The effects of each of the injected agents on most of the thermoregulatory parameters measured were clearly dose-dependent. The presence of a regular relationship between dose and thermoregulatory response does not prove that the injected agents acted on specific receptors. However, the absence of a highly irregular or bell-shaped dose-effect relationship is significant, for these latter functional relationships have been considered indicative of non-specific activity (Beckman, 1970; Bruinvels, 1970). (4) Intrathecal injection of small doses (0·0175–0·070 μmol) of the α-adrenergic agonist clonidine produced dose-dependent changes in thermoregulation similar to those induced by intrathecal NA. Moreover, intrathecal injections of a large dose (0·30 μmol) of *d*-NA, a NA enantiomer which is a weak agonist at noradrenergic receptors, caused little or no thermoregulatory effect. (5) In preliminary studies employing specific receptor antagonists, we have found that intrathecal pre-treatment with atropine greatly attenuates the hyperthermic effect of CCh injected intrathecally (R. M. LoPachin & T. A. Rudy, unpublished).

The spinal cord as the site of action for NA, 5-HT and CCh injected intrathecally

A drug injected into the lumbar subarachnoid space might be carried in the c.s.f. to supraspinal sites where drug-induced changes in thermoregulation could be mediated. However, previous work has shown that substances of widely varying molecular weight and lipid solubility (urea, morphine, naloxone) did not reach the supraspinal structures in appreciable concentration within the first 2 h after lumbar intrathecal injection (Yaksh & Rudy, 1976; Yeung & Rudy, 1980). As each of the substances employed in the present study elicited thermoregulatory effects within a few minutes after injection, it seems highly unlikely that these effects were mediated by a direct action on supraspinal structures. For 5-HT and CCh, an additional

argument against an action at supraspinal sites is the disparity in thermoregulatory effect evoked by these agents applied intrathecally and supraspinally. Intrathecal injections of these agents evoked hyperthermia,whereas 5-HT and CCh injected intraventricularly (I.V.T.) or intracisternally (I.C.) have generally been found to produce a fall in T_c (see reviews by Clark & Clark, 1980*a*, *b*).

An important consideration in any study of thermoregulation involving the central administration of a drug is the possibility that the injected drug produces its effects on temperature regulation by directly stimulating peripheral thermoeffectors subsequent to leakage of the agent from its central site of injection. Our results show that the thermoregulatory effects of intrathecal 5-HT and CCh are not produced by leakage to a peripheral site or sites of action; I.V. infusions of large doses of these agents did not alter T_c or T_{sk}.

In contrast, I.V. infusion of NA caused a fall in T_c associated with a very brief rise in T_{sk}. These results suggest that the hypothermic and vasodilatatory effects elicited by intrathecal NA might have been mediated, at least in part, by an action within the periphery rather than upon the spinal cord. Physiologically significant amounts of NA appear in the peripheral circulation following I.V.T., I.C. and intrahypothalamic (I.H.) injections (Mayer, Maickel & Brodie, 1960; Struyker-Boudier, Sweets, Brouwer & van Rossum, 1974; Buccafusco & Brezenoff, 1977), and it thus seems likely that NA injected into the spinal subarachnoid space also escapes into the circulation. Therefore, it is imperative to carry out additional studies on the sites mediating the thermoregulatory effects of NA injected intrathecally, and such investigations are in progress. However, based on evidence at hand, we believe it is reasonable to conclude tentatively that the hypothermia and tail skin vasodilatation elicited by intrathecal NA, especially by the lower doses, were mediated primarily by an action on the spinal cord. In the first place, intrathecal injections of NA consistently produced hypothermia, whereas I.V. infusions,although they reliably evoked transient vasodilatation, caused a fall in T_c in only 50% of the rats tested. Secondly, the tail skin vasodilatation which occurred after an intrathecal dose of NA was always greater in magnitude and much longer in duration than that which occurred after a comparable dose of NA given I.V. Thirdly, the magnitude of the hypothermic response elicited by I.V. doses of NA was significantly less than that produced by the same doses given intrathecally. This was true even when rats in which I.V. NA produced no fall in body temperature were excluded from the analysis. The importance of the second and third points made above is enhanced by the likelihood that not all of the intrathecally injected NA escaped into the circulation.

The largest intrathecal dose of NA examined in the present experiments (0·30 μmol) produced a hypothermia which was preceded by a brief rise in T_c. This initial hyperthermia was not produced by any of the lower intrathecal doses of NA tested and may be a product of a non-specific, high-dose effect on the spinal cord. However, a similar initial hyperthermic effect was seen in some of the rats infused I.V. with 0·10 μmol NA (the highest I.V. dose tested). It is possible, therefore, that this effect of NA is peripherally mediated, perhaps by activation of the noradrenergic receptors involved in the control of non-shivering thermogenesis.

Potential spinal cord sites of action of NA, 5-HT and CCh.

In the present investigation, the agonists studied were injected into the c.s.f.-filled space surrounding the lumbar spinal cord. Among the many possible neuro-anatomical substrates which might have been reached by the injected agents, three seem worthwhile discussing at the present time: (a) the dorsal horn, (b) spinal thermodetector units, and (c) the intermediolateral cell column (i.m.l.).

Research has suggested that bulbospinal noradrenergic and serotonergic fibres terminating in the dorsal horn modulate sensory transmission (Wall, 1967; Jordan, Kenshals, Martin, Haber & Willis, 1978; Headley, Duggan & Griersmith, 1978; Yaksh & Wilson, 1979; Reddy, Maderdrut & Yaksh, 1980). Moreover, results from two studies have indicated that cholinergic nerve terminals might influence sensory transmission in the dorsal horn (Weight & Salmoriaghi, 1966; Myslinski & Randic, 1977), although other research has not confirmed these findings (Headley *et al.* 1978). Thus, in theory the thermoregulatory changes induced by intrathecal injection of NA, 5-HT and CCh could be produced by an action of these agents within the dorsal horn.

As was mentioned previously, modulation of thermal afferent transmission through an effect on the dorsal horn should result in an apparent alteration in the thermoregulatory setpoint. Our findings in regard to the entire effect of 5-HT and the early portion of the CCh effect are commensurate with such a site and mode of action.

The apparent change in set point associated with the 5-HT effect and the early part of the CCh response could also have been produced by modulation of input from spinal thermodetector units. There exists good evidence that the thoraco-lumbar cord contains thermodetector neurones similar to those located in the rostral hypothalamus and preoptic region (Hensel, 1973; Lin & Chai, 1974; Simon, 1974), although the anatomical location within spinal tissues of these units remains to be determined. Thermal information from spinal thermosensitive structures is transmitted to supraspinal sites by the spinothalamic tract (Wunnenberg & Bruck, 1970; Simon & Iriki, 1971). Thus, thermodetector units probably synapse with dorsal horn interneurones or directly with spinothalamic cells. 5-HT, and possibly CCh, might modulate the input from spinal thermodetectors by acting on these synapses.

Because intrathecally injected NA produced hypothermia and vasodilatation which were frequently temporally dissociated and because the hypothermia was not associated with inhibition of shivering-like activity, we suggested earlier that this substance may have inhibited at the spinal level sympathetic outflow pathways controlling vasomotor tone and non-shivering thermogenesis. Preganglionic sympathetic neurones are located in the intermediolateral cell column (i.m.l.) of the spinal grey matter, and according to several researchers (Ryall, 1967; Coote & Macleod, 1974, 1977), bulbospinal noradrenergic fibres terminating in the i.m.l. inhibit the activity of these neurones. Moreover, it has been suggested that an action of clonidine at spinal sites mediates the observed sympathoinhibitory effects of this drug when administered by several central or peripheral routes (Sinha, Atkinson & Schmitt, 1973; Smith, 1974; Dhawan, Johri, Singh, Srimal & Viswesaram, 1975). Thus, intrathecally injected NA and clonidine might have caused hypothermia and vasodilatation by acting within the i.m.l.

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

the vasodilatation was a consequence of an action on i.m.l. units. However, this speculation is not supported by micro-ionophoretic data. Application of ACh to preganglionic sympathetic neurones in the i.m.l. did not alter their firing rate (DeGroat & Ryall, 1967).

With regard to 5-HT, we think an action of this agent on the i.m.l. is unlikely as the thermoregulatory effects of 5-HT are more compatible with a dorsal horn site of action. Furthermore, intrathecal 5-HT produced effects which are consistent with an increase in sympathetic outflow, whereas a sympatho-inhibitory role for bulbospinal 5-HT has been suggested by Coote & Macleod (1974) and by Neumayr, Hare & Franz (1974).

This work was supported by United States Office of Naval Research Contract N00014-75-C-0939. Preliminary reports of a portion of these findings appeared in *Fedn Proc.* **38**, 756 (1979) and *The Pharmacologist* **22**, 184 (1980). This work was done in partial fulfillment of requirements for the degree of Doctor of Philosophy, the University of Wisconsin (R.M.L).

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