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Evaluation of Neurotensin's Thermolytic Action by ICV Infusion with Receptor Antagonists and a Ca^{++} Chelator

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LEE, T. F., J. R. HEPLER AND R. D. MYERS. *Evaluation of neurotensin's thermolytic action by ICV infusion with receptor antagonists and a Ca^{++}* chelator. PHARMACOL BIOCHEM BEHAV **19**(3) 477–481, 1983.—To investigate the mechanisms responsible for the thermolytic action of neurotensin (NT), cannulae for intracerebroventricular (ICV) injection were implanted stereotaxically in Sprague-Dawley rats. Postoperatively, body temperature in the unrestrained rat was monitored continuously by a colonic thermistor probe. NT in a dose of 1.5–4.5 μg or CSF carrier vehicle was infused bilaterally in the cerebral ventricle (ICV) in a volume of 7.5 μl. With the rats kept at an ambient temperature of 22°C, NT given ICV produced a dose-dependent fall in core temperature of greater than 0.8°C. Pre-treatment of the animal's cerebral ventricle with amine receptor antagonists, phentolamine (20.0 μg), butaclamol (10.0 μg), methysergide (20.0 μg) or atropine (25.0 μg), all similarly infused ICV, failed to alter the hypothermia induced by NT. However, the calcium chelating agent, EGTA, given ICV in a dose of 4.0–8.0 μg blocked the thermolytic effect of NT on body temperature in a concentration-dependent manner. These results suggest that the central thermolytic action of NT is not mediated by catecholamine or other aminergic pathways which are implicated in the central mechanisms of thermoregulation. Rather, the peptide may act on a cellular process involving calcium activity in the hypothalamus, presumably to impair the maintenance of the animal's "set-point" for body temperature.

Neurotensin Monoamine theory EGTA Set-point Thermolytic response Hypothermia
Calcium ions Receptor antagonists Thermoregulation

MANY endogenous substances including neuroactive peptides, administered directly into the brain, exert an effect on body temperature [4,17]. However, nearly all of the observations obtained with peptides are limited in two respects. First, the functional meaning of a peptide-induced alteration in body temperature has not been elucidated [13]. Secondly, the mechanism of the thermolytic action of a given peptide [3] has not been integrated into one of the current theoretical views of the neurohumoral and ionic control systems in the CNS underlying the regulation of body temperature [21].

When injected centrally, neurotensin (NT) evokes a number of physiological and behavioral responses in different species [10,11]. For example, this tri-decapeptide given intracerebroventricularly (ICV) in the rat causes a fall in its body temperature [2] which is exacerbated in a cold environment [14]. Although this peptide may serve as a "neuromodulator" since its thermolytic action apparently can be mediated within the anterior hypothalamic, preoptic area [13], the fundamental mechanism for the effects of NT remains obscure. Based on results with receptor antagonists injected systemically, it has been suggested that other than dopamine, neither a monoamine nor acetylcholine is involved in the thermolytic action of NT [29]. This interpretation could be misleading because a given antagonist: (1) may not penetrate the blood-brain barrier [18]; (2) could exert its effect non-specifically on a peripheral thermoregulatory process [22]; or (3) lack specificity in terms of the class of receptor occupation. In relation to these points, phenoxybenzamine has been shown to act on several types of receptor [3]. Moreover, haloperidol given systemically even in moderately low doses can produce catalepsy in the rat [15].

The present study was undertaken to examine several alternative neuronal mechanisms in the brain which could be responsible for the reported hypothermia caused by NT. In this case, the procedure of ICV infusion was used not only to circumvent the blood-brain barrier [19] but also to eliminate the confounding effect on differentiated systems in the brain of a peripherally administered drug [22]. Further, in contrast to drugs used previously, more specific pharmacological antagonists of catecholamine and indoleamine receptors were used, namely, phentolamine, butaclamol and methysergide instead of phenoxybenzamine, haloperidol and PCPA. Finally, since several peptides are thought to disrupt thermoregulation by interfering with the animal's physiological "set-point" for body temperature [1,3], the relationship

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was investigated between the thermolytic action of NT and the molecular process in the brain believed to maintain the "set-point" for body temperature; i.e., the ratio between diencephalic sodium and calcium ions [18, 23, 26].

METHOD

Female Sprague-Dawley rats weighing 300 to 400 g were housed in individual cages at a room temperature of 21–22°C. They were maintained on a 12-hour light-dark cycle with water and Purina food pellets available ad lib.

Surgical Procedures

Each guide cannula for ICV infusion was cut from 23-gauge thin-walled stainless steel tubing to a length of 15.0 mm. Under sodium pentobarbital anesthesia (35 mg/kg IP), a guide tube was implanted bilaterally in each rat following procedures described earlier [16], and utilizing the following standard stereotaxic coordinates: AP=5.8 mm, Lat=1.5 mm, Hor=2.0 mm below the dura mater. The tip of each guide tube was beveled and positioned 1.0 mm above the lateral ventricle in order to minimize damage to the actual infusion site. A 27-gauge indwelling stylet was always kept in the cannula to prevent its occlusion.

After completion of the experiments, the precise anatomical location of the injector cannula was subsequently verified histologically according to standard anatomical procedures.

Temperature Measurements

The core temperature of the unrestrained rat was measured continuously by a YSI 401 thermistor probe inserted into the animal's colon to a depth of 4.0–6.0 cm. The probe was held in place by adhesive tape wrapped gently around the base of the tail. Colonic temperature was recorded every 15 min for at least 1.5 hr before an infusion was given and at five min intervals thereafter.

ICV Administration of Compounds

One week after surgery, each rat was tested with a standard test dose of 20.0 μg norepinephrine (NE) given ICV [28]. Only those animals which responded to NE with a fall in core temperature of more than 0.5°C were included for further study.

The following compounds were used: atropine sulfate (Fisher), butaclamol hydrochloride (Ayerst), ethyleneglycol-bis-N,N'-tetra-acetic acid (EGTA) (Sigma), methysergide bimaleate (Sandoz), neurotensin (Bachem), norepinephrine hydrochloride (Sigma) and phentolamine hydrochloride (CIBA-Geigy). Each drug was prepared immediately before an experiment began in a pyrogen-free artificial CSF containing Na^+ 127.7, K^+ 2.6, Ca^{++} 1.0, Mg^{++} 0.9 and Cl^- 132.1 mM [16]. To serve as an anti-oxidant, 0.1 mg/ml ascorbic acid was added to the solution of NE. Each test solution was always passed through a 0.22 μm Swinnex millipore filter and then infused bilaterally in a volume of 7.5 μl into the lateral ventricles by gravity flow. The receptor antagonists were similarly infused ICV, 15 min before the agonist, in doses which previously have been shown to block their respective agonist actions [5, 6, 17]. The doses in the Results section are expressed in terms of micrograms of the salt.

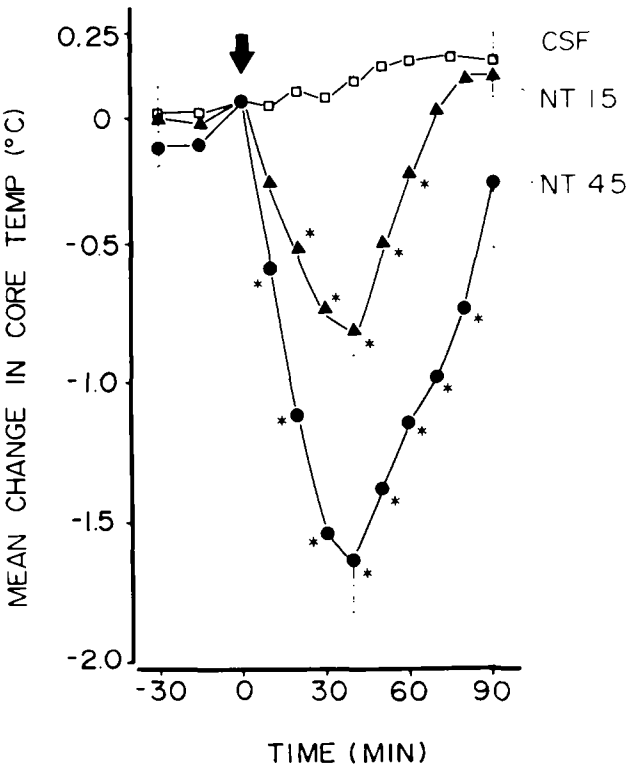

FIG. 1. Time course of the mean colonic temperature of the rat (n=5) in response to ICV infusion at zero hr of CSF or NT. Vertical bar denotes standard error at 45 and 90 min. Asterisk shows significant difference from CSF control ($p<0.05$, Student t-test).

Statistical Analysis

Comparisons between groups were made by either two-tailed Student t-tests or two-way ANOVA, and unless otherwise stated, a significant difference between groups was taken as $p<0.05$ [32]. For each comparison, the mean ±S.E. is presented as the index of the response.

RESULTS

ICV Infusion of NT

When the rat was kept at an ambient temperature of 22°C, an ICV infusion of artificial CSF exerted no significant effect on the animal's body temperature. Conversely, an ICV infusion of 1.5 μg NT caused an immediate fall in core temperature, as shown in Fig. 1, which reached a nadir of 0.85°C within 30–40 min after the infusion. Figure 1 illustrates the greater fall in the animal's temperature produced by NT when the dose was increased to 4.5 μg.

Effect of EGTA on Hypothermia Induced by NT Versus NE

To elucidate the effect of the central chelation of calcium on NT-induced hypothermia, 1.5 μg of the peptide was used as a submaximal test dose for this part of the study. As reported previously, an ICV infusion of EGTA causes a slight rise in the core temperature of the rat [24], i.e., 4.0 μg induces a rise of +0.24±0.15 (n=4) whereas 8.0 μg increased temperature by +0.37±0.14 (n=6). In neither case was the

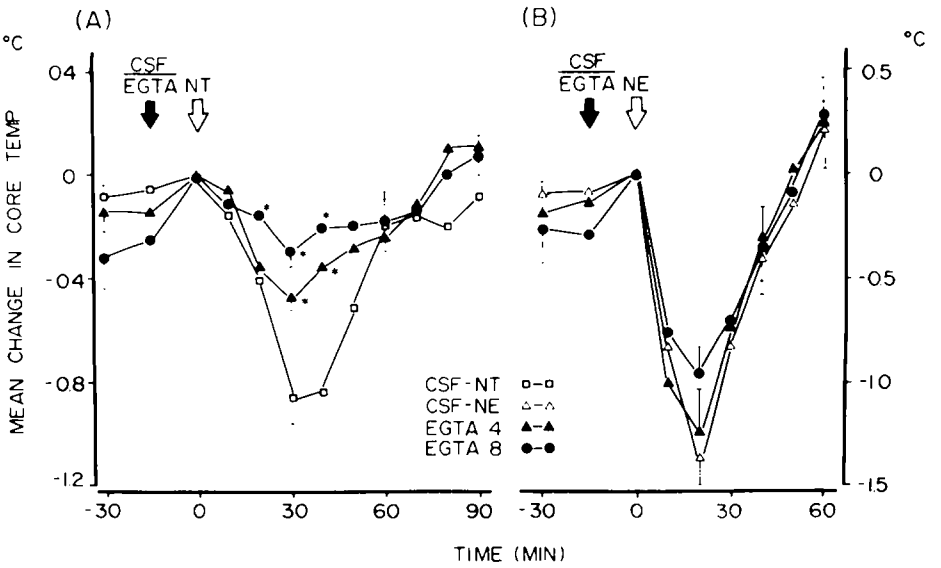

FIG. 2. Mean colonic temperature of the rat (n = 5) in response to ICV infusion of either: (A) 1.5 μg NT, or (B) 20 μg NE, after pre-treatment with either CSF or EGTA. Vertical bar indicates standard error. Each asterisk shows significant difference from CSF-pre-treated NT group ($p < 0.05$, Student t-test).

change significantly different from that of the CSF control. However, as shown in Fig. 2A, the hypothermia following the ICV infusion of NT was significantly attenuated in a dose-related manner by ICV pre-treatment with both 4.0 and 8.0 μg doses of EGTA infused 15 min earlier, $F(2,12)=6.16$, $p \leq 0.01$; dose × time interaction – $F(8,96)=3.50$, $p \leq 0.01$.

In agreement with previous findings [28], the ICV administration of 20 μg of NE induced a significant hypothermia in the rat, as illustrated in Fig. 2B. In contrast to the blockade of NT-hypothermia, EGTA infused ICV failed to prevent the NE-induced fall in the animal's body temperature. As depicted in Fig. 2B, only the higher dose of 8.0 μg of EGTA tended to retard slightly the magnitude of NE-hypothermia; however, this difference was not significant, $F(2,12)=0.18$; dose × time interaction $=F(5,60)=0.36$.

Receptor Antagonists and NT Hypothermia

Figure 3 presents a comparison of the mean maximal decline in the rats' body temperature in response to NT infused ICV after selected receptor antagonists were infused by the same route. Pre-treatment with either 10.0 μg butaclamol or 20.0 μg methysergide given ICV failed to alter the hypothermic response to NT. Even though a slight enhancement of the NT-induced decline in temperature was observed after ICV pre-treatment with 20.0 μg phentolamine or 25.0 μg atropine, the overal mean maximal change in core temperature was not significantly different from that of the CSF control (Fig. 3). As shown previously [25], ICV pre-treatment with these doses of the amine receptor antagonists alone produced no significant alteration in the body temperature of the rat when compared with CSF-treated controls.

Table 1 presents a temporal comparison at four 20-min intervals of the ICV effects of each of the receptor antagonists on NT-induced hypothermia. It is evident from the Table that all values denoting the average decline in the

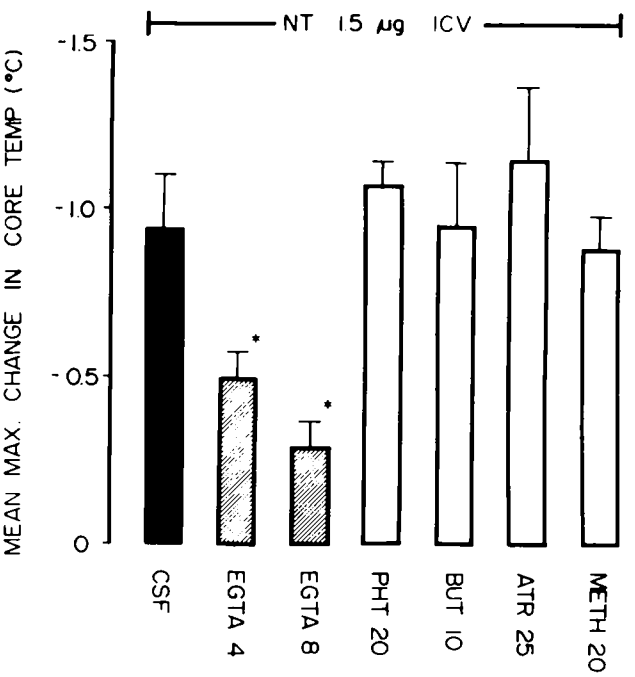

FIG. 3. Mean maximum change in core temperature (n = 5) after EGTA or four receptor antagonists on hypothermia induced by ICV infusion of 1.5 μg NT. Dose in μg follows compound's abbreviation. Standard errors are depicted on each vertical bar. Each asterisk denotes significant difference from CSF-pre-treated NT group ($p < 0.05$, Student t-test).

TABLE 1

HYPOTHERMIA RECORDED AT 20 MINUTE INTERVALS AFTER ICV INFUSION OF TRANSMITTER RECEPTOR ANTAGONISTS FOLLOWED BY 1.5 μg NT GIVEN ICV IN THE RAT†

Treatment + 1.5 μg NT	Post-Injection Time (in Minutes) 20	40	60	80
CSF	−0.51 ± 0.04	−0.84 ± 0.20	0.29 ± 0.12	−0.19 ± 0.14
phentolamine (20 μg)	−0.85 ± 0.10*	−1.04 ± 0.09	−0.48 ± 0.18	−0.10 ± 0.19
butaclamol (10 μg)	−0.53 ± 0.13	0.88 ± 0.18	−0.53 ± 0.23	0.05 ± 0.09
atropine (25 μg)	−0.80 ± 0.17	1.08 ± 0.30	−0.51 ± 0.23	−0.26 ± 0.09
methysergide (20 μg)	−0.68 ± 0.09	0.73 ± 0.10	−0.19 ± 0.11	0.06 ± 0.13

†Each value represents mean decline in baseline core temperature recorded at the time of NT infusion.

*Significant difference from CSF controls, $p<0.05$ (Student t-test).

rats' body temperature, following the respective ICV infusion of each compound, was in each case within 0.2°C.

DISCUSSION

Even though the central administration of NT produces a hypothermic response in the rat kept at room temperature [2,14], in most cases the dose of NT typically used is much higher than that required to elicit analgesia [7]. This raises the possibility that the hypothermia caused by NT may be secondary to a decrease in activity, sedation or a more general impairment of autonomic function. In order to evaluate the underlying mechanism, a lower dose of NT is thus required to eliminate these potentially confounding side-effects. One way to achieve this is to provide a sufficient volume of carrier infusate, as used in this study, to ensure that NT is dispersed widely throughout the brain's ventricular system. Since no postmortem enlargement of the ventricular spaces was observed on our study, this was a valid recourse in that: (1) the solution was infused by gravity flow rather than by forced ICV injection; and (2) the volume of infusate was well below the limit of the ventricular lumen [19]. Thus, in terms of dilution and concentration factors [18], the efficacious dose used in the present study was actually much lower than that used previously.

Following the introduction of the monoamine theory of thermoregulation in the early 1960's [9], additional classical neurotransmitters including acetylcholine were implicated in the central mechanisms of thermoregulation [17]. From the results of the present study, however, it is clear that NT-induced hypothermia is apparently not mediated by central muscarinic, α-adrenergic or serotonergic receptors believed to mediate the neuronal pathways for heat loss and heat production [21]. That is, the central pre-treatment with an antagonist which impairs transmission in these neuronal "circuits" does not alter the thermolytic response to NT. The slight enhancement of NT-induced hypothermia observed in the rat pre-treated ICV with phentolamine or atropine may have been due to their respective inhibition of the neurons believed to comprise the heat production system in the hypothalamus [27].

In disagreement with a previous study [29], NT-induced hypothermia was not enhanced after ICV pre-treatment with a specific dopamine receptor antagonist, butaclamol. In fact, the intensity of the decline in the rats' body temperature was essentially identical to that observed in the absence of this dopaminergic antagonist. The discrepancy may be due to the fact that the enhancement of NT-induced hypothermia occurred only when a dose of haloperidol given systemically was used which in itself caused a substantial reduction in body temperature [29]. Moreover, it is well documented that the stimulation of central dopamine receptors causes a fall in core temperature of the rat [8]. Therefore, it is difficult to interpret an enhancement of hypothermia by haloperidol except on the basis of relative non-specificity of the drug used, the dose administered, or its route of administration—peripheral.

Overall, it is thus clear that the NT-induced decline in core temperature does not depend on any of the acknowledged neurotransmitter systems in the brain that play a functional role in thermoregulatory processes [21]. In contrast, the central chelation of Ca^{++} ions by EGTA given ICV inhibits the thermolytic effect of NT in a dose-related manner. This attenuation could not simply be ascribed to the thermogenic property of EGTA [24] since the hypothermia induced by NE is not significantly altered by EGTA given similarly. One parsimonious explanation for the ameliorative action of EGTA is that the hypothermia elicited by NT in fact requires an endogenous neurotransmitter other than one of several that have been investigated in the present study. By reducing the availability of Ca^{++} ions on neuronal membranes or terminals of the efferent pathway delegated to heat dissipation, the thermolytic effect of NT could be prevented. An alternative explanation is that NT interferes with the functional mechanism responsible for the maintenance of the body temperature "set-point," which is postulated to involve the intrinsic ratio between calcium and sodium ions in the hypothalamus [26]. Since the established "set-point" temperature can be reset by EGTA [23], it is possible that NT thus fails to elicit its effect after EGTA pre-treatment because the "set-point" is already shifted. Since the posterior hypothalamus is the locus of the cation set-point mechanism , this region should be exceptionally sensitive to NT. Although this area has not been examined in the only study published to date on anatomical mapping of NT's effect on temperature [13], the finding that the hypothermic response to NT is unaffected by an electrolytic lesion of the anterior hypothalamus [30] would favor this view.

Recent observations made in our Laboratory suggest that NT does possess poikilothermic attributes. Exposure of the

rat to a warm ambient temperature immediately after NT is administered ICV prevents or attenuates the typical hypothermia [12]. In relation to this is the observation that the poikilothermic effect of other compounds such as alcohol or a surgical anesthetic is either blocked or reversed by the central chelation of calcium ions by EGTA again infused ICV [24,25]. Conversely, antagonists of monoamine receptors do not alter drug-induced poikilothermia, which corresponds to the findings of the present investigation.

Therefore, taking all of these observations together, it is likely that NT does not possess any regulatory role in the sensory signaling, cellular integration, and efferent processing of thermal information required for the adaptive defense of the body's set temperature. Whether or not this peptide has any functional role in the mechanism which in most mammals establishes the body temperature set-point at 37°C also remains equally uncertain.

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