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Neurotensin: Distribution of Hypothermic Response in Mammalian and Submammalian Vertebrates

ARTHUR J. PRANGE, JR., CHARLES B. NEMEROFF, GARTH BISSETTE, PAUL J. MANBERG, ALBERT J. OSBAHR, III, GORDON B. BURNETT, PETER T. LOOSEN AND GARY W. KRAEMER*

*Biological Sciences Research Center Department of Psychiatry and The Neurobiology Program, University of North Carolina, School of Medicine Chapel Hill, NC 27514 and *Primate Laboratory and Wisconsin Psychiatric Research Institute, University of Wisconsin School of Medicine, Madison, WI 53206*

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PRANGE, A. J., JR., C. B. NEMEROFF, G. BISSETTE, P. J. MANBERG, A. J. OSBAHR, III, G. B. BURNETT, P. T. LOOSEN AND G. W. KRAEMER. *Neurotensin: distribution of hypothermic response in mammalian and submammalian vertebrates*. PHARMAC. BIOCHEM. BEHAV. 11(5) 473–477, 1979.—Neurotensin (NT), an endogenous tridecapeptide (pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu-OH), is a potent hypothermic agent after central administration in the mouse and rat. The purpose of the present investigation was to evaluate the effect of NT on thermoregulatory processes in a variety of mammalian and non-mammalian vertebrates: bluegill, frog, lizard, pigeon, ground squirrel, woodchuck, golden hamster, rabbit, guinea pig, gerbil, mouse, rat and monkey. All species except monkey were tested in two ambient environmental temperatures, 23°C and 4°C, except poikilotherms. Animals were injected intracisternally with microgram quantities of NT or vehicle, and body temperature was periodically assessed over a 2 hr period. NT did not induce a significant alteration in body temperature in any of the poikilotherms studied (bluegill, frog, and lizard). At 23°C NT produced a significant hypothermic response in the mouse, rat, gerbil, and monkey with no effect observed in the pigeon, rabbit, guinea pig, golden hamster, ground squirrel or woodchuck. At 4°C, NT produced a significant decrease in body temperature in the mouse, rat, gerbil, guinea pig and golden hamster with no effect evident in the pigeon, rabbit, ground squrrel or woodchuck. Species known to respond to cold by increasing metabolic rate (e.g. mouse and rat) appear to be most responsive to NT. The hypothermic activity of NT in a variety of mammals suggests that the peptide may play a role in thermoregulation.

Neurotensin Thermoregulation Neuropeptides Hypothermia Hibernators

NEUROTENSIN (NT) is a tridecapeptide, pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu-OH [5,4]. Radioimmunoassay and immunohistochemical techniques have been used to identify NT in gut [10,16] and brain [16, 21, 22]. When injected peripherally it causes vasodilation, hypotension, increased vascular permeability and transient cyanosis [1, 4, 5]. After peripheral injection certain endocrine effects also have been noted, including hyperglycemia [2, 7, 13] and the release of a variety of anterior pituitary hormones [4, 12, 19]. When injected directly into the cerebroventricular system, NT causes hypothermia in rats and mice [1, 3, 14]. Recently intracisternally (IC) administered NT has been reported to exert antinociceptive effects in mice [8]. Diminished locomotion and extension of barbiturate narcosis have also been noted after central administration, but these effects may be attributable to the hypothermic action of NT [14]. Given peripherally, even large doses of the peptide exert no effects on body temperature [14] or nociception [8], suggesting that the blood-brain barrier effectively excludes this substance.

Since the discovery of the hypothermic effect of NT, studies have revealed that the C-terminal end of the molecule is essential for this action [11]. NT-induced hypothermia appears not to depend upon the integrity of brain cholinergic, noradrenergic, or serotonergic neural systems [15,18]. To further explore the possible physiological role of NT in thermoregulation we have studied its effects after central administration in a variety of species, and this is the subject of the present report.

METHOD

Representatives from 13 species were selected for study (Table 1), at least one from each of the vertebrate classes. (The species tested were as follows: bluegill, Lepomis macrochirus; frog, Rana pipiens; lizard, Anolis carolenensis; pigeon, Columba domestica; ground squirrel, Citellus tridecalineatus; woodchuck, Marmota monax, golden hamster, Mesocricetus auratus; rabbit, Oryctolagus cuniculus; guinea pig, Cavia porcellus; gerbil, Gerbillus gerbillus;

TABLE 1

THE EFFECT OF INTRACISTERNAL NEUROTENSIN ON RECTAL TEMPERATURE IN WARM (23° C) AND COLD (4° C) AMBIENT TEMPERATURES

Species	Sex	Body Wt. (kg, approx.)	Brain Wt. (g, approx.)	Dose (μg)	Volume (μl)	Warm (23° C) no. times N	Warm (23° C) no. times sig.	Warm (23° C) NT effect	Cold (4° C) no. times N	Cold (4° C) no. times sig.	Cold (4° C) NT effect
Poikilotherms											
Non-mammals											
Non-hibernators											
Fish											
Bluegill	M,F	0.06	0.1	30	10	—	—	?	6	0	No
Amphibian											
Frog	M,F	0.06	0.2	30	10	8	0	No	8	0	No
Reptile											
Lizard	M,F	0.004	0.05	15	5	16	0	No	7	0	No
Homeotherms											
Non-mammal											
Non-hibernator											
Bird											
Pigeon	M,F	0.55	2.5	100	10	6	0	No	6	0	No
Mammals											
Obligatory hibernators											
Rodents											
Ground Squirrel	M,F	0.10	3.5	30	10	7	0	No	5	0	No
Woodchuck	M,F	5.5	15.0	100	20	3	0	No	4	0	No
Permissive hibernator											
Rodent											
Golden hamster	M	0.14	1.0	30	10	6	0	No	5	3	Yes
Non-hibernators											
Rodents											
Rabbit	M	3.5	8.0	300	30	5	0	No	5	0	No
Guinea pig	M	0.35	4.0	30	10	6	0	No	6	3	Yes
Gerbil	M,F	0.05	1.0	10	10	6	4	Yes	7	3	Yes
Mouse	M	0.03	0.5	10	10	7	4	Yes	6	4	Yes
Rat	M	0.30	2.0	30	10	6	4	Yes	6	4	Yes
Primate											
Monkey	M	4.0	80.0	150	10	4	3	Yes	—	—	?

Groups of animals were injected intracisternally with neurotensin or vehicle (0.9% NaCl, pH 7.5). Thereafter they were individually housed at ambient temperature (23° C) or, in other experiments, in a cold room (4° C). Rectal temperature was assessed immediately after injection (0 time) and at 30 minute intervals for two hours. "Cold" experiments in lizards and frogs were conducted at 15° C because of their intolerance to colder temperatures. Bluegills were subjected to gradually cooling water (see text). Monkeys were studied only in a warm environment; bluegills only in a cold environment. In each experiment significant differences occurred at most times (3–4) or at none. Student's *t*-test (two-tailed interpretation) was used to assess statistical significance. When for a given species a positive effect of neurotensin is shown, the table lists the minimal effective dose; when a null effect is shown, the largest dose employed is listed.

Swiss-Webster mouse, Mus musculus; Sprague-Dawley rat, Rattus norvegius; rhesus monkey, Macaca mulatta.) All animals were bred in captivity except woodchucks, ground squirrels and bluegills. All animals, domesticated or wild, were obtained from commercial sources. Hibernators were studied at times when they would not naturally be hibernating: ground squirrels, early October; woodchucks, September; hamsters, August.

Animals, except woodchucks, ground squirrels, and bluegills, were housed locally for at least 7 days before experimentation and received food and water ad lib. Woodchucks were studied the day after their capture and the evening after their arrival in the laboratory.Ground squirrels were studied the day after their arrival. Bluegills were studied about four hours after they were received. All experiments were conducted about midday, except as noted in the case of woodchucks. Animals were given light ether anesthesia, except lizards, frogs, and bluegills, which received no anesthesia, and monkeys, which received ketamine, 10 mg/kg, intravenously. All animals were injected IC, except bluegills, which were injected intracerebrally, as is commonly done in laboratory goldfish [20]. In all species our ability to perform accurate IC injections was tested by using Evans Blue dye in a few pilot animals. After IC injection of the dye the brain was removed from the calvarium, rapidly frozen on dry ice and sectioned on a cryostat. Tissue sections were observed for ventricular penetration of the dye. To facilitate injections in woodchucks, it was necessary to make a longitudinal midline incision and retract the massive neck extensors.

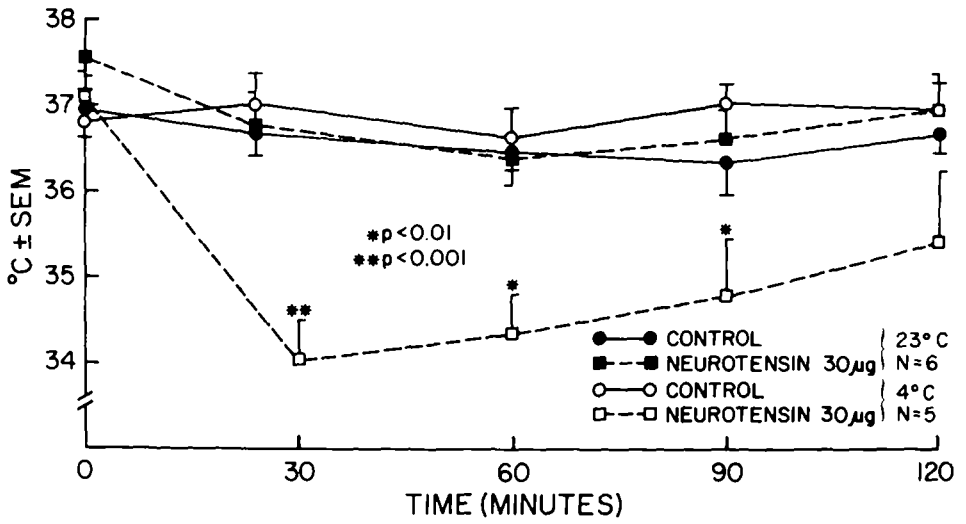

FIG. 1.The effect of intracisternal (IC) neurotensin (30 μg) or vehicle (0.9% NaCl, pH 7.5) on rectal temperature of adult male golden hamsters in a warm (23°C) and a cold (4°C) environment. See text for methods utilized. (Student's *t* test, two-tailed for unmatched samples).

For each species equal numbers of control and experimental animals were used. Control animals were handled, anesthetized and injected in a manner similar to experimental animals, except that the injectate was 0.9% sterile NaCl, which also served as the vehicle for NT. Immediately before each experiment NT was freshly prepared in vehicle. The peptide was generously supplied by Dr. Jean Rivier of the Salk Institute, La Jolla, CA. Various doses and injectate volumes were used depending on the species under study (Table 1). We purposely erred on the side of avoiding false negative results, and thus some rather small animals were given comparatively large doses of NT. Most species were studied at more than one dose level in the same condition (23°C, 4°C). Dose-response relationships had been established in mice and rats [1,14], and were not explored in the present study. The present work, then, was addressed to the qualitative question of the distribution of the hypothermic response to centrally administered NT in typical representatives of the vertebrate subphylum.

After IC injections animals were housed in individual cages without water, food or bedding for two hours, though bluegills, of course, were kept immersed. In warm experiments the ambient temperature was 23°C; in cold experiments, 4°C. Rectal or cloacal temperature was determined by use of a thermistor probe (Yellow Springs Instrument Company) within two minutes after injection and therafter at 30, 60, 90 and 120 min.

Poikilotherms were studied to determine if NT would affect the rate of change of body temperature in response to transfer from a warmer to a cooler environment. Cold experiments in poikilotherms were specifically designed. Lizards and frogs, even uninjected, lost body temperature so rapidly when placed at 4°C, that there was no opportunity, as it were, for NT to show an effect. Thus in cold experiments lizards and frogs were transferred to a room at 15°C immediately after injection, where they lost body temperature gradually. Bluegills were more difficult to study. When even untreated animals were suddenly transferred to water as little as 3°C cooler than their customary water, they acted stunned and then moribund. To avoid this profound effect, fish were transferred, after injection, to individual wire mesh cages in a large aquarium, the water temperature of which was identical to that of their storage tank, 23°C. As the water was mechanically circulated (and aerated) ice was added in such fashion as to lower the temperature to 13°C in 30 min at a fairly constant rate. Other members of the team determined core temperature of the fish at 6, 12, 18, 24 and 30 min. after cooling of the water began.

In order to examine the effects of stress on the response to NT, 12 adult male albino rats (300 g) were restraint-stressed in wire mesh for three hours [17] prior to study. Groups of animals were then injected IC with NT (30 μg) or vehicle and placed in a cold (4°C) environment as described above.

In a related series of experiments, rabbits were handled (gentled) daily for 1 week, a lateral ventricular cannula was implanted, and they were further gentled daily for 3 more weeks. In this manner, we attempted to reduce the degree of stress produced by our experimental procedure. Following this period of gentling, these rabbits were injected with 300 μg NT intracerebroventricularly and rectal temperatures were assessed at 4°C as described above.

RESULTS

Figure 1 displays the results obtained in the golden hamster. These results are shown to provide an example of the basis on which the generalizations in Table 1 are obtained. It can be seen that NT (30 μg) failed to exert a hypothermic effect when animals were kept at room temperature. In a cold environment, on the other hand, the same dose produced a pronounced hypothermic effect. As for all other species, except bluegills, (five time points), there were four time points at which differences could have occurred. In Table 1 we have recorded that for golden hamsters at no time were there significant differences between experimental and control animals in the warm (23°C) experiment, while at three times there were such differences in the cold (4°C) experiment.

Table 1 summarizes the results of the various experi-

ments. Most species, as stated, were studied at two dose levels in both conditions, though never did the larger dose produce an effect when the smaller had failed. When both doses produced an effect the larger produced a greater effect. In Table 1, when we have reported a positive effect, data pertaining to the smaller dose are shown; when a null effect, the larger dose. Whether a hypothermic effect occurred can be discerned from the columns labelled "number of times significant". When active in the warm NT was active in the cold (rat, mouse and gerbil), though the converse did not hold (golden hamster and guinea pig). Within species equal doses produced greater differences from controls in the cold than in the warm (data not shown) as in previous reports [1, 11, 14].

NT was active in at least one condition in all mammals except rabbit and in obligatory hibernators (woodchuck and ground squirrel). It was inactive in both conditions in these three species. In the hamster, considered a permissive hibernator [9], NT was active only in the cold. NT exerted no discernible effect in the pigeon or in the three species of poikilotherms.

In rats which were restraint-stressed, NT-induced hypothermia was offset by less than 40%, an effect which was not statistically significant. In addition, rabbits which had been handled daily for 4 weeks remained unresponsive to intracerebroventricular injection of 300μg of NT.

DISCUSSION

It appears that centrally administered NT exerts a hypothermic effect in mammals except hibernators. A clear exception is the rabbit, in which NT does not cause hypothermia in either the warm or the cold. This is probably not the result of inadequate dosage, for rabbits were given as much as twice the dose given monkeys. Rabbits appear to be unresponsive to other effects of NT as well. For example, NT administered intravenously does not produce hypotension in this species (Marvin Brown, The Salk Institute, personal communication).

Stress conceivably could have masked a NT-induced hypothermic response and thus accounted for the negative results obtained in some species. However, in rats, restraint-stress did not abolish responsiveness to NT. Conversely, in rabbits, the only non-hibernating mammal totally unresponsive to NT in both the warm and cold, reduction of stress by daily handling did not result in responsiveness to NT. Thus attempts to eliminate stress did not convert a non-responsive species to a responsive one. These data indicate that stress probably does not account for the failure of the rabbit to show a hypothermic response to NT.

At the present time, the brains of only four species, rat, guinea pig, rabbit and calf, have been studied for the presence of NT [5, 6, 21, 22]. By immunoreactive techniques, it has been found in all. We are ignorant of species in which NT has been sought but not found. Thus data pertaining to the distribution of NT in brains of various species is too sparse to be helpful in understanding the pattern of responsiveness to NT.

A factor which may account for species differences in responsiveness to NT is species differences in response to cold exposure [18]. Species which have been shown to respond to cold by increasing their metabolic rate (e.g. mouse, rat) appear to be most responsive to NT; species which do not significantly increase metabolic heat production (e.g. rabbit, ground squirrel) at the temperature conditions we used are unresponsive to NT. Guinea pig provides an interesting interplay of these two variables. Precht *et al.*[18] showed that this species is intermediate between mouse and rabbit in metabolic response to cold. We found that guinea pig is intermediate in hypothermic response to NT, the response failing to occur at room temperature but becoming manifest in the cold. These correlations suggest that NT exerts its hypothermic effect by inhibiting heat production. This inhibition, occurring only after central administration, may involve, among other possibilities, an attenuation of sympathetic activity or an inhibition of shivering.

Clearly in poikilothermic species NT does not produce a hypothermic effect. It is possible that false negative results were obtained in these species in the cold simply because they lost temperature so rapidly that induced changes could not be observed, though precautions were taken to avoid this possibility. If this is the case, however, it could not account for our failure to find effects in a warm environment when such were readily observed in other species. Cold per se may have been more stressful to small animals than large ones, contributing to the pattern of results. This could not have been a factor in the warm, however, and it was in the warm that our largest species, monkey, was shown to be NT responsive.

The broad but specific distribution of NT hypothermic response in a wide variety of species suggests that this endogenous peptide, along with many other factors, may play a role in thermoregulation and its disorders.

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