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# Role of Neurotensin in Radiation-Induced Hypothermia in Rats

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KANDASAMY, S. B., HUNT, W. A., AND HARRIS, A. H. Role of Neurotensin in Radiation-Induced Hypothermia in Rats. *Radiat. Res.* 126, 218-222 (1991).

The role of neurotensin in radiation-induced hypothermia was examined. Intracerebroventricular (ICV) administration of neurotensin produced dose-dependent hypothermia. Histamine appears to mediate neurotensin-induced hypothermia because the mast cell stabilizer disodium cromoglycate and antihistamines blocked the hypothermic effects of neurotensin. An ICV pretreatment with neurotensin antibody attenuated neurotensin-induced hypothermia, but did not attenuate radiation-induced hypothermia, suggesting that radiation-induced hypothermia was not mediated by neurotensin. © 1991 Academic Press, Inc.

## INTRODUCTION

Exposure to ionizing radiation causes changes in core body temperature. This effect depends partly upon the species of animal, with hyperthermia occurring in cats, rabbits (1), and humans (2), and a biphasic response (i.e., a fall in temperature followed by a rise) in monkeys (3). In rats, the direction of the temperature change is dose dependent with hyperthermia occurring when radiation doses are less than 15 Gy and hypothermia occurring when doses are greater than 20 Gy. In rats, the temperature response appears to be centrally mediated because irradiation of the head alone causes these effects, while irradiation of the trunk only does not (4, 5).

The chemical mediators identified thus far include prostaglandins (5) and histamine (5), while serotonin has been shown not to be involved (4). It is not known if histamine release is a primary response to irradiation or secondary to the release of other substances, such as neurotensin, liberated in the cascade of events following radiation injury.

Neurotensin, an endogenous tridecapeptide found in the central nervous system, particularly in hypothalamus, caudate nucleus, globus pallidus, putamen, nucleus accumbens and amygdala (6-9), and gastrointestinal tract (10), acts as a neurotransmitter or a neuromodulator (11). Specific neurotensin binding sites have been identified in the midbrain (6, 12), and interactions between neurotensin and

dopamine have been shown (13). Central administration of neurotensin produces a variety of behavioral and physiological effects, including the stimulation of histamine release (14). One of the more potent effects of neurotensin is the induction of hypothermia after intracisternal or intraventricular administration (15-17). The purposes of this study were to investigate the role of neurotensin in radiation-induced hypothermia and to elucidate the mechanisms involved in neurotensin-induced hypothermia.

## METHODS

**Drugs.** The drugs used were neurotensin (Sigma Chemical Co., St. Louis, MO), neurotensin antibody (Accurate Chemical and Scientific Corporation, Westbury, NY), disodium cromoglycate (Fisons Corporation, Bedford, MA), mepyramine maleate (Mallinckrodt Inc., St. Louis, MO), cimetidine (Smith Kline and French Laboratory, Philadelphia, PA), ketamine hydrochloride (Parke-Davis, Detroit, MI), xylazine (Hayer Lockhart, Shawnee, KS), and acepromazine (Ayerst Laboratories, NY). Neurotensin, neurotensin antibody, mepyramine, and disodium cromoglycate were dissolved in sterile, nonpyrogenic saline. Cimetidine was dissolved in 0.1 M of 1 N HCl and diluted to the final volume with saline.

**Animals.** Male Sprague-Dawley Crl:CD(SD)BRD rats (Charles River Breeding Laboratories, Kingston, NY) weighing 200-300 g were used in these experiments. Rats were quarantined on arrival and screened for evidence of disease by serology and histopathology. The rats were housed individually in polycarbonate isolator cages (Lab Products, Maywood, NJ) on autoclaved hardwood contact bedding (Beta Chip, Northeastern Products Corp., Warrensburg, NY), and were provided commercial rodent chow (Wayne Rodent Blok, Continental Grain Co., Chicago, IL) and acidified water (pH 2.5 using HCl) *ad libitum*. Animal holding rooms were kept at  $21 \pm 1^\circ\text{C}$  with  $50 \pm 10\%$  relative humidity on a 12-h light/dark cycle with no twilight.

**Radiation exposure.** Rats were placed in clear plastic well-ventilated containers for approximately 5 min before irradiation or sham exposure. The animals were then exposed bilaterally to  $\gamma$  rays using a  $^{60}\text{Co}$  source at a rate of 20 Gy/min to a total dose of 50 Gy. Prior to the experiment, the dose rate at the midline of an acrylic rat phantom was measured using a 0.5-cc tissue-equivalent ionization chamber manufactured by Exradin, Inc. The dose rate at the same location with the phantom removed was measured using a 50-cc ionization chamber fabricated at AFRRRI. The ratio of these two dose rates, the tissue-air ratio, was used to determine the doses for animals receiving routine experimental exposures, in this experiment, the tissue-air ratio was 0.93. All ionization chambers have calibration factors traceable to the National Institute for Standards and Technology. Dosimetry measurements were performed following the AAPM Task Group 21 Protocol for the Determination of the Absorbed Dose from High-Energy Photon and Electron Beams (18).

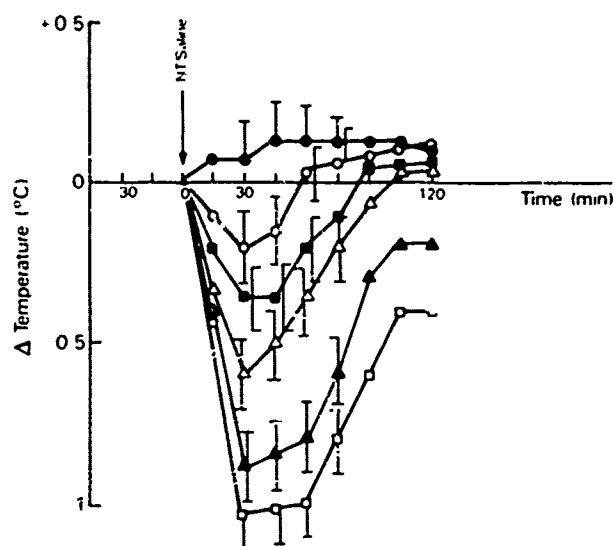


FIG. 1. Changes in rectal temperature of rats induced by intracerebroventricular (ICV) injection of neurotensin (NT): 1  $\mu$ g (○), 3  $\mu$ g (■), 5  $\mu$ g (Δ), 10  $\mu$ g (▲), 20  $\mu$ g (□), and saline (●). Each point represents the mean  $\pm$  SE of observations of five animals. Zero on the ordinate represents the temperature at the time of injection.

**Central administration of drugs.** Rats were anesthetized with 1 ml/kg of a mixture of ketamine (50 mg/kg), xylazine (5 mg/kg), and acepromazine (1 ml/kg) given intramuscularly, and were placed in a rat stereotaxic apparatus (David Kopf Instruments, No. 320). A single cannula was inserted aseptically into the lateral ventricle according to coordinates derived from the atlas of Pellegrino *et al.* (19): 0.8 mm posterior to bregma, 2.5 mm lateral. The cannula was lowered until cerebrospinal fluid rose in the cannula. Dental acrylic was used to secure the cannula. Animals were allowed to recover for 1 week before they were used for experiments. After the end of an experiment, injection sites were verified histologically. Injections and irradiations were performed at the same time of day (0900) to avoid diurnal variations in temperature. The antagonists (neurotensin antibody, disodium cromoglycate, mepyramine, and cimetidine) were given 30 min before the radiation and neurotensin were administered.

**Measurement of body temperature.** All experiments were performed at a room temperature of  $22 \pm 1^\circ\text{C}$ . The animals were placed in restraining cages 1 h before starting the experiments, and body temperature was measured every 15 min for 2 h with thermistor probes (YSI series 700, Yellow Springs Co., Inc., Yellow Springs, OH) inserted approximately 6 cm into the rectum and connected to a datalogger (Minitrend 205). After each experiment, all animals were killed immediately with an overdose of carbon dioxide by inhalation.

**Statistics.** Statistical evaluations were performed using analysis of variance with a significance level of  $P < 0.05$ . Intergroup comparisons were made using Tukey's test (20).

## RESULTS

An ICV administration of 1–20  $\mu$ g of neurotensin induced hypothermia in a dose-dependent manner (Fig. 1). An ICV pretreatment with 0.25–1.0 mg of neurotensin antibody attenuated the hypothermia induced by ICV admin-

istration of 5  $\mu$ g of neurotensin, but did not reduce the hypothermia produced by 50 Gy of ionizing radiation (Fig. 2). Figure 2 also shows that administration of neurotensin antibody alone had no effect on body temperature. An ICV administration of the mast cell stabilizer disodium cromoglycate did not change the body temperature of control animals but did block, in a dose-dependent manner, the hypothermia produced by the ICV injection of 5  $\mu$ g neurotensin (Fig. 3). Mepyramine and cimetidine are specific  $H_1$  and  $H_2$  receptor antagonists, respectively (21). Mepyramine (100–300 ng, ICV) or cimetidine (100–300 ng, ICV) was administered before neurotensin to examine the role of histaminergic  $H_1$  and  $H_2$  receptors in neurotensin-induced hypothermia. Previous studies (4, 5) indicate that the same doses of mepyramine and cimetidine are specific  $H_1$  and  $H_2$  receptor antagonists, respectively, and did not change the body temperature in control animals. Both mepyramine and cimetidine, which are found to antagonize histamine-induced hypothermia (4, 5) attenuated neurotensin-induced hypothermia (Fig. 4).

## DISCUSSION

Ionizing radiation induces either hyperthermia or hypothermia depending upon the dose; temperature changes appear to be centrally mediated (4, 5). Histamine has been implicated in the actions of ionizing radiation, including hypotension, reduced cerebral blood flow, and performance decrement (22). Furthermore, concentrations of histamine in circulating blood have been elevated in humans undergoing radiation therapy (23) as well as in dogs and monkeys (24–26) following radiation exposure. Histamine is present in a high concentration in the hypothalamus (27, 28), and is localized in nerve terminals (29), suggesting that it may act as a central neurotransmitter. Also, histamine is involved in many physiological functions, including thermoregulation, and could underlie radiation-induced hypothermia (4, 5, 30).

Histamine is stored in mast cells throughout the body (31), including the brain (32, 33), and neurotensin has been used to stimulate histamine release (34–38). When injected into the lateral cerebral ventricle, neurotensin induced a dose-dependent hypothermia in rats and mice (15–17), confirming previous results. Neurotensin is contained in the normal cerebrospinal fluid (39, 40), and may play a role in thermoregulation, because central administration of neurotensin decreases colonic temperature in rodents (15–17), but is ineffective following peripheral administration (15). In addition, Muraki *et al.* (41) reported a decrease in the level of neurotensin-like immunoreactivity in the cerebrospinal fluid of children with febrile aseptic meningitis,

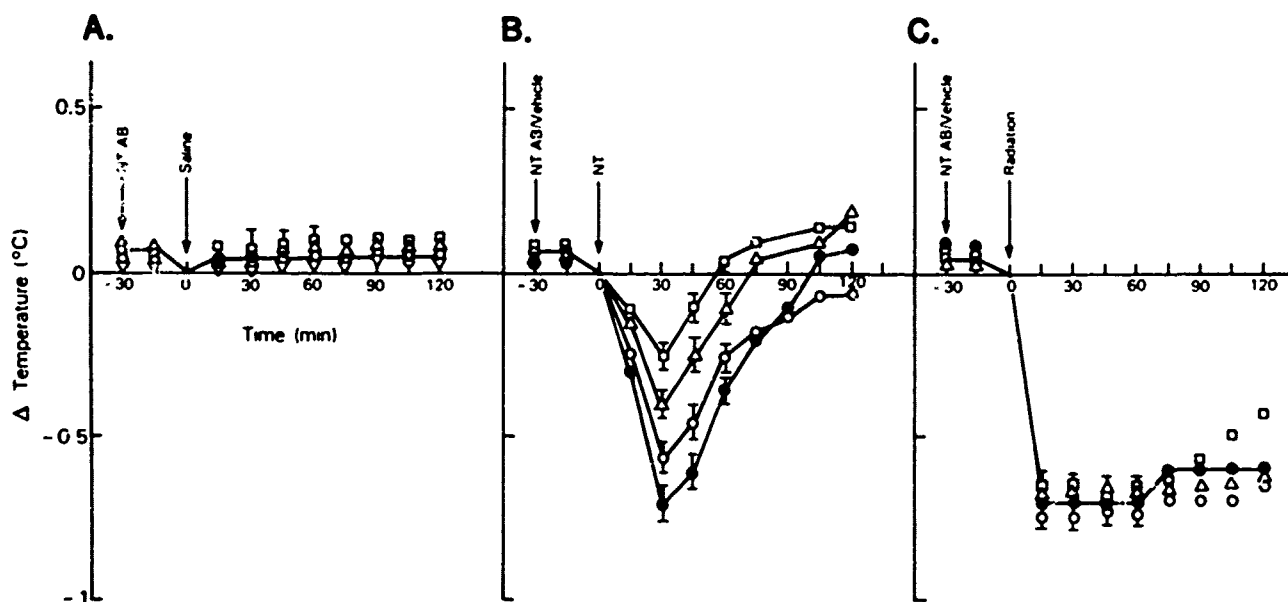


FIG. 2. Effect of neurotensin antibody (NT-AB), ICV, on NT-or radiation-induced hypothermia. (A) Nonirradiated controls given 25  $\mu\text{g}$  (O), 50  $\mu\text{g}$  ( $\Delta$ ), or 100  $\mu\text{g}$  ( $\square$ ) NT-AB; (B) 5  $\mu\text{g}$  of NT alone ( $\bullet$ ) or in the presence of 25  $\mu\text{g}$  (O), 50  $\mu\text{g}$  ( $\Delta$ ), or 100  $\mu\text{g}$  ( $\square$ ) NT-AB; (C) 50 Gy irradiation alone ( $\bullet$ ) or in the presence of 25  $\mu\text{g}$  (O), 50  $\mu\text{g}$  ( $\Delta$ ), or 100  $\mu\text{g}$  ( $\square$ ) NT-AB. Each point represents the mean  $\pm$  SE of observations of five animals. Zero on the ordinate represents the temperature at the time of second injection.

suggesting an important role for neurotensin in thermoregulation.

Disodium cromoglycate is a potent inhibitor of the immunological release of chemical mediators secreted from

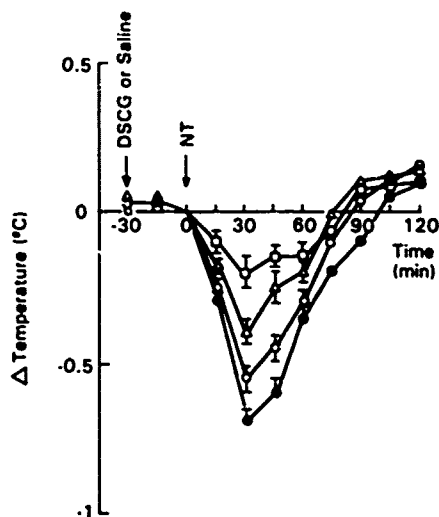


FIG. 3. Effect of disodium cromoglycate (DSCG), ICV, on NT-induced hypothermia. The 5  $\mu\text{g}$  of NT alone ( $\bullet$ ) or in the presence of 100 ng (O), 300 ng ( $\Delta$ ), or 500 ng ( $\square$ ) DSCG. Each point represents the mean  $\pm$  SE of observations of five animals. Zero on the ordinate represents the temperature at the time of second injection.

mast cells (21). It has been reported that neurotensin stimulates the release of histamine *in vivo* (37, 38, 42), and pretreatment with disodium cromoglycate inhibits the neurotensin-induced release of histamine (34, 36, 37, 43). The attenuation of neurotensin-induced hypothermia by disodium cromoglycate and histamine  $\text{H}_1$  and  $\text{H}_2$  receptor antagonists in this study suggests that neurotensin-induced hypothermia was mediated by histamine.

There are no specific antagonists available to antagonize neurotensin-induced hypothermia; therefore, specific antibodies to neurotensin were used to eliminate endogenous neurotensin, because they would bind and inactivate the neuropeptide (43–45). Researchers have reported that the centrally administered neurotensin antibody inhibits neurotensin-induced nociception and hypothermia (44, 45). In our experiments, pretreatment with neurotensin antibody attenuated neurotensin-induced hypothermia, but had no inhibitory effect on radiation-induced hypothermia, suggesting that neurotensin may not be involved in radiation-induced hypothermia. Although Cockerham *et al.* (46) found a nonsignificant increase in plasma neurotensin levels following exposure to ionizing radiation, because neurotensin is degraded rapidly, they think tissue levels might be significantly increased, causing the release of histamine from mast cells (46). In our study, pretreatment with neurotensin antibody, which attenuated neurotensin-induced hypothermia, did not reduce radiation-induced hypothermia.

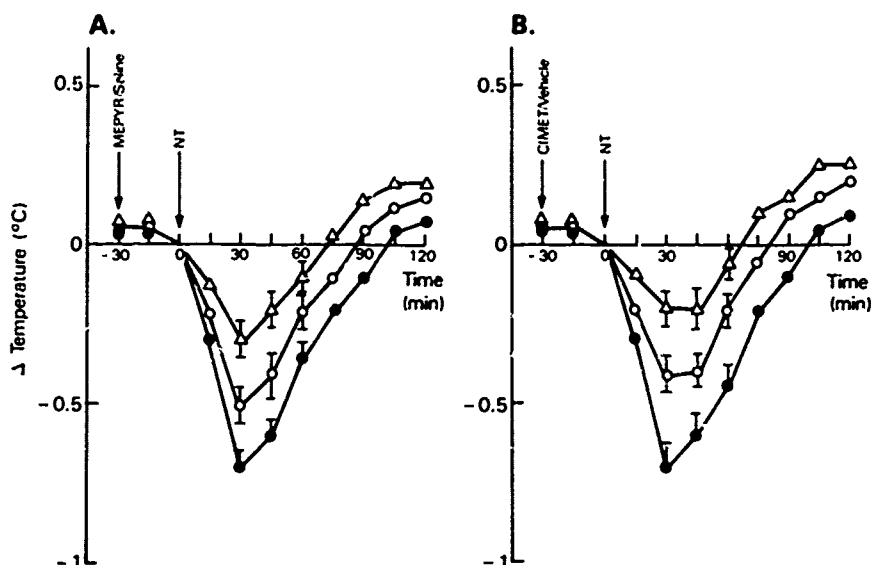


FIG. 4. Effect of mepyramine (MEPYR), ICV, or cimetidine (CIMET), ICV, on NT-induced hypothermia. (A) 5 µg of NT alone (●) or in the presence of 100 ng (○) or 300 ng (Δ) mepyramine. (B) 5 µg of NT alone (●) or in the presence of 100 ng (○) or 300 ng (Δ) cimetidine. Each point represents the mean  $\pm$  SE of observations of five animals. Zero on the ordinate represents the temperature at the time of second injection.

Although neurotensin-induced hypothermia is mediated by histamine, we could not determine if radiation-induced hypothermia is mediated by endogenous neurotensin.

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