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Role of Brain Neurotransmitters on Neurotensin-Induced Gastric Cytoprotection

DANIEL E. HERNANDEZ,*† DONALD A. STANLEY,† J. ALLEN MELVIN AND ARTHUR J. PRANGE, JR.†‡

**School of Veterinary Medicine, North Carolina State University*
4700 Hillsborough Street, Raleigh, NC 27606
and †Biological Sciences Research Center, ‡Department of Psychiatry
University of North Carolina School of Medicine, Chapel Hill, NC 27514

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HERNANDEZ, D. E., D. A. STANLEY, J. A. MELVIN AND A. J. PRANGE, JR. *Role of brain neurotransmitters on neurotensin-induced gastric cytoprotection.* PHARMACOL BIOCHEM BEHAV 22(4) 509–513, 1985.—We have reported previously that intracisternal (IC) administration of neurotensin (NT) prevents stress-induced gastric ulcers in rats. This effect of NT appears to be mediated by the central nervous system because peripheral (IV) NT is totally ineffective. The present study sought to clarify the central mechanism of the cytoprotective effect of NT by utilizing pharmacological treatments which alter the function of brain neurotransmitter systems. Pretreatment with intracerebroventricular (ICV) administration of agonists and antagonists of acetylcholine (ACh), γ-aminobutyric acid (GABA), and serotonin (5-HT) receptors or with an anti-opiate (naloxone) agent did not significantly alter NT-induced cytoprotection. However, pretreatment with ICV haloperidol, a dopamine (DA) receptor antagonist, totally blocked NT's cytoprotective effect. In addition, pretreatment with methylphenidate, a DA receptor agonist, produced cytoprotection similar to IC NT. These data indicate that NT-induced cytoprotection is not mediated by 5-HT, GABA, ACh (muscarinic) receptors, or endogenous opiate systems, but suggest interactions between brain DA systems and NT.

Neurotransmitters Neurotensin Dopamine Stress ulcers

A considerable body of evidence suggests that brain neuropeptides thought to modulate central neurotransmission processes may play a role in gastrointestinal homeostasis [12]. This contention is supported by immunohistochemical studies which have revealed a dual distribution of these peptides in brain areas involved in autonomic regulation and in peripheral tissues immediately relevant to contractile and secretory functions of the gastrointestinal tract [19]. Compatible with this interpretation is the growing body of evidence suggesting that several brain-gut peptides affect gastric secretion and that they may, consequently, be involved in the pathogenesis of gastric and duodenal ulcerations.

This former view is based upon recent findings which have shown that some neuropeptides, such as thyrotropin-releasing hormone, increase acid secretion [23], whereas others, in contrast, including bombesin and β-endorphin, inhibit gastric acid secretion [21]. Interestingly, many of these gastric-anti-secretory peptides have been shown to exert a salutary effect against gastric and duodenal ulcers produced by a variety of chemical and psychobehavioral factors [7, 10, 22].

In our early work with neurotensin (NT), an endogenous brain and gastrointestinal tridecapeptide, we reported in descriptive and mechanistic terms that intracisternal (IC) administration of NT totally prevents cold-restraint stress (CRS)-induced gastric ulcerations in rats [17]. This protective effect of NT, contrary to our original hypothesis, is not mediated by inhibition of gastric acid secretion but requires an intact gut prostaglandin synthetic pathway [1]. In subsequent studies, we have found evidence that the sympathoadrenomedullary axis plays an intermediary role in both the expression of NT-induced cytoprotection and in the development of CRS-induced gastric ulcers [8,18].

In recent studies we have shown that the cytoprotective effect of central NT is relatively specific because only β-endorphin and bombesin, as previously reported [22], are cytoprotective, whereas in contrast, a number of other peptides tested including substance P, somatostatin, gastrin, CCK-8, met- and leu-enkephalin, and bradykinin are not [10].

An interesting observation is that NT is cytoprotective only after central (IC) but not peripheral (intravenous) administration, suggesting that this effect of NT is originally mediated by the central nervous system (CNS).

This study was conducted to investigate the involvement of selected brain neurotransmitter systems on the cytoprotective effect of NT by using the CRS model.

METHOD

Adult male Sprague-Dawley rats (180–220 g) from Zivic-Miller (Cincinnati, OH) were housed in a controlled environment animal facility and fed laboratory chow with water ad lib. Rats were habituated to the animal facility for a minimum of 7 days before experimental use. All rats were deprived of food, but not water, 24 hr before the experiments. After this, they were lightly anesthetized with ether and injected intracerebroventricularly (ICV) with the tech-

nique of Popick [20], intracisternally (IC), as previously described [9], or intraperitoneally (IP). The basic experimental design utilized 4 groups ($n \geq 10$ rats/group) treated in the following manner: (1) saline pretreatment ICV + saline IC, (2) saline pretreatment ICV + NT 30 μg IC, (3) drug pretreatment ICV + saline IC, and (4) drug pretreatment ICV + NT 30 μg IC. In a separate set of experiments, rats were pretreated with IP saline or naloxone (2 mg/kg) 1 hr before IC saline or NT (30 μg).

All central (ICV or IC) injections were given in 10 μl vehicle (sterile 0.9% NaCl), except for haloperidol which was dissolved in 0.3% tartaric acid.

Both the dose administered and the interval between the time of drug (or vehicle) pretreatment and IC administration of NT (or vehicle) was determined from the available literature to achieve optimal drug effect [2, 4, 6, 11, 14, 15, 16, 24]. The following drugs were utilized (followed by the dose, route of administration and pretreatment interval): atropine (25 μg, ICV, 30 min); carbachol (1 μg, ICV, 30 min); bicuculline (1 μg, ICV, 30 min); naloxone (2 mg/kg, IP, 60 min); methysergide (1 μg ICV, 30 min); cyproheptadine (1 μg, ICV, 30 min); haloperidol (5 μg, ICV in 0.3% tartaric acid, 30 min); muscimol (50 ng, ICV); and methylphenidate (80 μg, ICV, 60 min). Drug doses were calculated as salts.

Immediately after the IC injections with vehicle (10 μl 0.9% NaCl) or NT (30 μg), all rats were restrained in wire mesh and placed supine in a cold (4°C) room for 3 hr. This regimen of physical restraint plus subsequent exposure to a cold environment has been reported elsewhere to reliably produce gastric ulcers in the glandular portion of the rat stomach [5, 8, 22]. After 3 hr of cold-restraint stress (CRS), the rats were killed by decapitation. The stomach was removed immediately, and the gastric mucosa was exposed by cutting along the lesser curvature. The incidence of gastric ulcers was assessed by direct observation under a dissecting microscope (10×) by a trained observer unaware of the treatment regimen and the severity data was obtained by counting the number of ulcers as previously described [1, 8, 10, 17].

Neurotensin was obtained from Bachem (Torrance, CA), haloperidol from McNeil Laboratories (Fort Washington, PA), and naloxone from Endo Laboratories (Garden City, NY). Methylphenidate was a gift from Ciba-Geigy Corporation (Summit, NJ). Atropine sulphate, cyproheptadine, and muscimol were obtained from Sigma Chemical Company (St. Louis, MO). Bicuculline was purchased from Vega Biochemicals (Tucson, AZ) and methysergide from Sandoz Pharmaceuticals (East Hanover, NJ). Statistical analysis was performed using the Kruskal-Wallis test for multiple comparisons of nonparametric data and the Dunnett's test for multiple comparison of parametric data. A p-value of 0.05 or less was considered to represent significant differences between treatment groups.

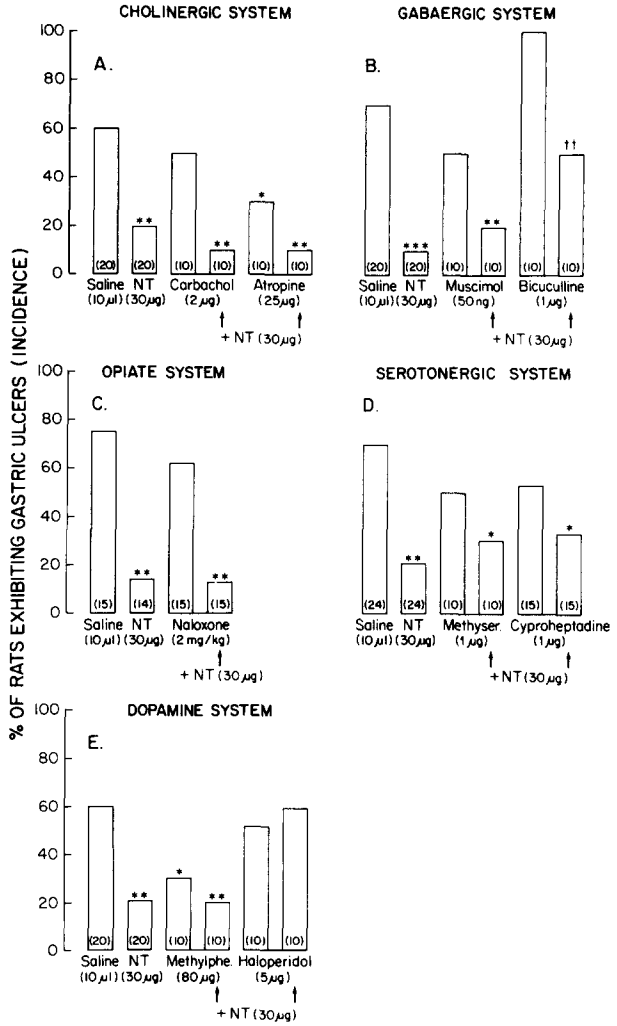

FIG. 1. Effect of pharmacological manipulations of brain neurotransmitter systems on neurotensin-induced gastric cytoprotection against cold-restraint stress gastric ulcers in rats. Groups of 24 hr food-deprived rats were drug-pretreated with ICV agonists and antagonists of cholinergic (A), GABAergic (B), opiate (C), serotonergic (D), and dopamine (E) systems and then injected IC with either vehicle (10 μl of 0.9% NaCl) or NT (30 μg). For details, see the text. The numbers inside the bars refer to the number of rats in each treatment group. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ when compared to saline-treated controls (Kruskal-Wallis test). $^{\dagger\dagger}p<0.01$ when compared to bicuculline-treated rats (Kruskal-Wallis test).

RESULTS

The results presented in Fig. 1 and Table 1 show the effect of ICV administration of cholinergic (muscarinic), GABA, 5-HT, and DA receptor agonists and antagonists, and of IP administration of an opiate receptor antagonist on the cytoprotective activity of IC administered NT.

In confirmation of previous findings [1, 10, 17], IC NT (30 μg) produced a significant ($p<0.01$) reduction of gastric ulcer incidence and severity (Fig. 1A–E, Table 1). Intracerebroventricular pretreatment with either carbachol (a cholinergic muscarinic receptor agonist) (Fig. 1A), muscimol (a GABA agonist) (Fig. 1B), naloxone (an opiate receptor antagonist administered IP) (Fig. 1C), methysergide (a 5-HT receptor agonist) (Fig. 1D), or with cyproheptadine (a 5-HT receptor antagonist) (Fig. 1D) did not affect NT-induced gastric cytoprotection. In addition, none of these agents had any effect when administered alone as evidenced by a similar gastric ulcer incidence and severity when compared to saline-treated controls (Table 1).

Of interest, however, were our findings obtained with atropine pretreatment (Fig. 1A, Table 1), which produced cytoprotection similar to IC NT, and with bicuculline (a GABA receptor antagonist), which only partially antago-

TABLE 1

EFFECT OF AGONISTS AND ANTAGONISTS OF Ach, GABA, OPIATES, 5-HT, AND DA RECEPTORS ON NEUROTENSIN (NT)-INDUCED GASTRIC CYTOPROTECTION AGAINST STRESS GASTRIC ULCERS IN RATS

Treatment	Severity of Gastric Ulcers (No. of ulcers/rat ± S.E.M.)
Saline	2.1 ± 0.4
NT (30 μg)	0.5 ± 0.2*
Carbachol (2 μg)	1.8 ± 0.3
Carbachol + NT	0.3 ± 0.1*
Atropine (25 μg)	0.5 ± 0.3*
Atropine + NT	0.2 ± 0.2*
Saline	2.2 ± 0.1
NT (30 μg)	0 ± 0†
Muscimol (50 ng)	1.8 ± 0.3
Muscimol + NT	0.1 ± 0.1†
Bicuculline (1 μg)	8.4 ± 1.9†
Bicuculline + NT	1 ± 0.3*
Saline	1.8 ± 0.7
NT (30 μg)	0.4 ± 0.3*
Naloxone (2 mg/kg, IP)	0.9 ± 0.3
Naloxone + NT	0.2 ± 0.1†
Saline	2.4 ± 0.3
NT (30 μg)	0.7 ± 0.2*
Methylsergide (1 μg)	1.4 ± 0.7
Methylsergide + NT	0.8 ± 0.5*
Cyproheptadine (1 μg)	2.0 ± 0.4
Cyproheptadine + NT	0.5 ± 0.2†
Saline	3 ± 1.1
NT (30 μg)	0.1 ± 0.1†
Methylphenidate (80 μg)	0.6 ± 0.1*
Methylphenidate + NT	0.8 ± 0.3*
Haloperidol (5 μg)	1.8 ± 0.6
Halopridol + NT	1.4 ± 0.4

*$p<0.05$, †$p<0.01$ when compared to saline-treated controls (Dunnett's test for multiple comparisons). Number of rats, treatments and route of administration same as indicated in Fig. 1. In the combination studies the doses are the same as when the drugs were administered singly.

nized the cytoprotective effect produced by IC NT (Fig. 1B). Although the incidence of gastric ulcers was higher in animals pretreated with bicuculline than in saline controls, this difference did not reach statistical significance (Fig. 1B). However, bicuculline-treated animals had a significantly ($p<0.01$) higher severity than the saline controls (Table 1).

Finally, the results of pretreatment with DA receptor agonists and antagonists are illustrated in Fig. 1E and Table 1. Blockade of DA receptors with haloperidol completely antagonized NT-induced cytoprotection. By contrast, DA receptor stimulation with ICV methylphenidate was as effective as central NT in producing a substantial reduction of stress gastric ulcers.

DISCUSSION

The results of this study confirm and extend previous findings that NT, administered IC, produces a significant reduction of CRS-induced gastric ulcers in rats [10,17]. The cytoprotective effect of central NT is apparently not mediated by interactions with muscarinic cholinergic, serotonergic, or endogenous opiate systems because pretreatments with selected agonists and antagonists which affect neurotransmission in these brain circuits did not significantly alter the cytoprotective response to IC NT.

An interesting observation was that blockade of cholinergic muscarinic receptors with ICV atropine, like NT, was cytoprotective. This finding corroborates the work of others [6], but suggests that an interaction between NT and central cholinergic systems is unlikely, at least in this paradigm, because neither receptor stimulation with carbachol nor receptor blockade with atropine had any appreciable effect on NT-induced gastric cytoprotection.

Of interest were our results obtained with bicuculline pretreatment which produced a partial antagonism of NT-induced cytoprotection. It should be noted that bicuculline alone significantly increased gastric ulcer severity (Table 1). In addition, IC NT significantly reduced the incidence and severity of gastric ulcers when compared to bicuculline-treated rats. Central (ICV) administration of the GABA

agonist, muscimol, alone or with NT, was totally ineffective. These results, which suggest that GABA receptors may only partially mediate NT-induced gastric cytoprotection, do not exclude the possibility that central GABAergic systems may be involved in gastrointestinal homeostasis. Levine *et al.* [11] have shown that muscimol increases gastric secretion in pylorus-ligated rats, and this effect is blocked by bicuculline, a GABA agonist.

The negative findings obtained with pharmacological manipulations of serotonin and opiate systems resonate with previous results suggesting that many of the CNS-dependent effects of NT, including NT-induced hypothermia and NT-induced analgesia, are not mediated by serotonin receptors or release of endogenous opiates [14]. In fact, in more recent work [3], we have found that co-administration of NT and leucinal, an aminopeptidase inhibitor which potentiates leu-enkephalin and β-endorphin-induced analgesia, does not affect the analgesic response to IC NT in the hot-plate test in mice. The results presented in this report further substantiate the lack of involvement of endogenous opiates on the central effects of NT.

Evidence does exist, however, that endogenous opiate peptides may play a role in the maintenance of gastrointestinal mucosal integrity. Morley *et al.* have found evidence that blockade of opiate receptors with naltrexone induces cytoprotection. Peripheral met-enkephalin, in contrast, was shown to be ulcerogenic [13]. In a previous report, we found that IC β-endorphin exerts a salutary effect against stress gastric ulcers in rats [10], and the cytoprotective properties of opiate peptides have been recently confirmed by another group [4].

Finally, as shown in Fig. 1E, haloperidol pretreatment totally antagonized NT-induced cytoprotection, and indirect stimulation of DA receptors with ICV methylphenidate produced cytoprotection similar to NT.

These data are consistent with other studies [15,16], suggesting an interaction between NT and brain DA systems. For example, it has been shown that NT, like neuroleptic drugs (all of which are DA receptor blockers), induces muscle relaxation, catalepsy, and potentiation of the sedative effects of ethanol and barbiturates. In addition, NT-induced hypothermia is exaggerated by impairment of central DA systems [14].

Recent neurochemical studies have shown that IC or ICV NT increased DA turnover in several brain areas, including nucleus accumbens and striatum [25]. Whether these brain loci are related to stress ulcer formation is the subject of current research.

In a previous report, we provided evidence that NT-induced cytoprotection is mediated by α- and β-receptors, therefore suggesting that the sympathetic (adrenergic) division of the autonomic nervous system plays an intermediary role in the expression of NT-induced gastric cytoprotection [18].

This study has shown that certain neurotransmitter systems (i.e., DA, GABA, ACh) may be involved in the pathogenesis of stress-induced gastric ulcers and suggest that endogenous peptides, like NT, may express their cytoprotective properties through interactions with brain DA circuits.

Although the data furnished in this report provide new information concerning NT-induced cytoprotection, a full description of its mechanism of action awaits further clarification.

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