EFFECT OF ACUTE VERAPAMIL TREATMENT ON COLD RESTRAINT-INDUCED GASTRIC LESIONS IN RATS WITH LESIONED NUCLEUS BASALIS MAGNOCELLULARIS

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SUMMARY

Our recent studies suggested that there were no differences in the number and type of cold restraint-induced gastric lesions (petechiae and erosions) between control and nucleus basalis magnocellularis (NBM)-lesioned rats. Having in mind that calcium antagonists can prevent the development of gastric stress ulcer in control rats, the aim of the present study was directed to compare the effect of verapamil in the prevention of cold restraint-induced gastric lesions in control and NBM-lesioned rats. Therefore, 30 min before the stress, control (intact control and sham-operated) rats as well as rats with bilateral electrolytic lesions of NBM were treated with saline (1 ml/kg s.c.) or verapamil (1.0, 2.5, 5.0 and 10.0 mg/kg s.c.) and exposed to cold restraint for 2 hr. Verapamil, in doses of 5.0 and 10.0 mg/kg, significantly decreased the number and length of gastric erosions ($p < 0.05$) in control rats, while in NBM-lesioned rats, the same doses of verapamil significantly decreased the number of gastric petechiae ($p < 0.01$). Since the antiulcer treatment with verapamil express different effects in the prevention of the two types of gastric lesions in control and NBM-lesioned rats, it can be concluded that in NBM-lesioned rats there may exist an alteration of the mechanisms involved in stress-induced gastric petechiae and erosions.

KEY WORDS: Cold restraint, Nucleus basalis magnocellularis, Rats, Stress ulcer, Verapamil

INTRODUCTION

The origin of cholinergic hypothesis of learning and memory deficits in Alzheimer's disease (AD) is based on loss and/or atrophy of cholinergic neurons in the nucleus basalis of Meynert (1,2). Therefore, experimental lesions of the homologous nucleus in the rat, the nucleus basalis magnocellularis (NBM), has been considered as an animal model of AD (3).

There is little information in the literature attempting to correlate dementia and gastroduodenal ulcer in humans (4,5,6). However, these few studies were unable to detect any

significant correlation between gastroduodenal ulcer and dementia.

Although one of our studies showed that there were no differences in cold restraint-induced gastric lesions between control and NBM-lesioned rats (7), futher results had shown that some differences did exist (8). Namely, this recent study confirmed that there were no differences in ulcer intensity between individually stressed control and NBM-lesioned rats, but suggested that group-stressed control rats expressed significantly lower intensity of stress-induced gastric lesions in comparison to group-stressed NBM-lesioned rats.

Previous studies have shown that calcium channel blocking agents, extensively used in the treatment of cardiovascular diseases, express significant protection in several models of stress-induced gastric lesions in rats. However, the antiulcer action of calcium antagonists can not be generalize, since their effects depend of the type of calcium antagonist as well as of the experimental model used for the production of gastric stress ulcer (9,10,11,12). On the other hand, it has been demonstrated that verapamil has a significant effect in the ameloration of cognitive and non-cognitive dysfunction in rats with lesioned nucleus basalis magnocellularis - NBM (13,14,15). Since verapamil can prevent the development of stress ulcer in control rats, the present study was carried out to determine if verapamil can prevent the development of cold restraint-induced gastric lesions in NBM-lesioned rats.

MATERIAL AND METHODS

Experimental animals

The present study was carried out on adult male Wistar rats (300-320 g). The rats were housed in groups of 5 to a plastic cage in standard laboratory conditions (room temperature of 20 ? 1?C, 30% humidity, 12/12 hr light/dark cycle) with food and tap water available ad libitum. The animals were divided in the following groups: intact control (IC), sham-operated (SO) rats and rats with lesioned NBM. The rats from each group were treated with either saline or verapamil. Nine rats were assigned to each group.

Surgery

The rats were anesthetized with sodium pentobarbital (35 mg/kg, i.p.). Bilateral electrolytic NBM lesions were made by using a direct current of 1.0 mA passed through a unipolar stainless steel electrode (0.15 mm in diameter) for 30 s by means of a Grass S88 stimulator (7). The coordinate for the NBM were 1.3 mm posterior to bregma, 2.2 mm from midline on each side and 6.5 mm ventral from dura (16). In SO animals the electrodes were only lowered into the NBM without turning on the current. The recovery period after operation was ten days.

Cold restraint

The rats were kept in individual cages with wire mesh bottoms (to prevent coprophagy)

and deprived of food but not water, during 24 hr before the stress.

In order to be stressed, the rats were placed into individual plastic restraint boxes. The size of the boxes was 18 x 5 x 7 cm. The length of the restraint boxes was adjusted by a sliding door. The restrained rats were exposed to the cold (4?C) for 2 hr (17). Two hours after cold restraint started, the rats were slightly etherized and sacrificed by decapitation. The stomach was removed, opened along the greater curvature and lightly rinsed with tap water. After that, the stomach was carefully swabbed, fixed between two glass plates (6 x 8 x 0.3 cm) and examined under an illuminated magnifier (x 3). The number of petechiae and erosions as well as the length of erosions were considered as stress ulcer parameters. The erosion size (mm) was determined by measuring each erosion along its greatest length (7).

Drugs

Verapamil hydrochloride (Sigma, St. Louis, MO) was dissolved in physiological saline immediately before use. Verapamil (1.0, 2.5, 5.0 and 10.0 mg/kg) or saline (1 ml/kg) were given subcutaneously, 30 min before the stress was started.

Histology

The position of NBM lesions was examined according to the previously described procedure (7).

Statistical analysis

All data are expressed as means ? S.E.M. Since the data did not show a normal distribution, statistical comparisons were performed using the nonparametric tests: Friedman and Kruskal-Wallis analysis of variance tests as well as the Mann-Whitney U-test. The level for statistical significance were $p < 0.05$.

RESULTS

Since there were no statistically significant differences in cold restraint-induced gastric lesions among IC and SO, the data of these two groups were pooled and reported as control group data. There were no significant differences in the number of petechiae and erosions as well as in the length of the erosions between control and NBM-lesioned rats injected with saline and exposed to cold restraint. The Friedman analysis of variance test showed significant differences between verapamil treated control rats and verapamil treated NBM-lesioned rats, in relation to the number of petechiae ($p < 0.01$), but not in relation to the number and length of erosions. The Kruskal-Wallis test showed that in NBM-lesioned rats treated with verapamil there are significant differences in the number of petechiae ($p < 0.01$) while in verapamil treated control rats the significant differences were obtained in relation to the number and length of the erosions ($p < 0.05$). Post hoc Mann-Whitney test showed that verapamil in doses of 5.0 and 10.0 mg/kg significantly reduced the number and the length of gastric erosions in control rats ($p < 0.05$ vs. saline treated control rats) (Table 1.).

Table 1. Effect of verapamil (VER) on cold restraint-induced gastric petechiae and erosions in control rats

Treatment (mg/kg)	No. of petechiae	No. of erosions	Length of erosions (mm)
Saline (1 ml/kg)	12.2 ? 1.7	3.9 ? 1.2	6.3 ? 1.5
VER (1.0)	10.0 ? 3.3	2.2 ? 0.7	3.2 ? 1.3
VER (2.5)	19.8 ? 4.6	2.2 ? 1.2	4.1 ? 2.1
VER (5.0)	16.2 ? 5.4	1.2 ? 0.6*	1.6 ? 0.8*
VER (10.0)	16.1 ? 2.8	1.0 ? 0.4*	1.2 ? 0.6*

The results are presented as mean ? S.E.M. * $p < 0.05$ vs saline treated group.

Table 2. Effect of verapamil (VER) on cold restraint-induced gastric petechiae and erosions in NBM-lesioned rats

Treatment (mg/kg)	No. of petechiae	No. of erosions	Length of erosions (mm)
Saline (1 ml/kg)	15.0 ? 3.6	2.9 ? 1.0	4.7 ? 1.6
VER (1.0)	12.4 ? 2.7	3.3 ? 1.1	4.7 ? 1.7
VER (2.5)	10.7 ? 2.4	3.1 ? 1.3	5.5 ? 2.5
VER (5.0)	7.9 ? 3.2*	1.3 ? 0.6	2.7 ? 1.3
VER (10.0)	5.6 ? 1.6*	1.4 ? 0.6	2.0 ? 0.9

The results are presented as mean ? S.E.M. * $p < 0.01$ vs saline treated group.

However, the same doses of verapamil, significantly reduced the number of petechiae but not the number and length of erosions in NBM-lesioned rats (vs. saline treated NBM-lesioned rats) (Table 2.).

DISCUSSION

One of our recent studies (7) was conducted to investigate the effect of NBM lesions on the development of gastric stress ulcer in male Wistar rats, individually exposed to cold restraint. This work indicated that the bilateral NBM lesions by itself did not induce

development of gastric ulcer. Moreover, there were no differences in the type and intensity of gastric lesion induced by cold restraint between control animals and NBM-lesioned rats. In a similar fashion, the present study suggests that there were no significant differences in the production of stress ulcer between control rats and NBM-lesioned rats, both treated with saline. However, a significant higher level of ulcer index in group stressed NBM-lesioned rats in comparison to group stressed control rats (8) indicated that social interaction was highly disrupted in NBM-lesioned rats. These results indicated that the adaptation processes in rats exposed to cold restraint could be modified by the lesion of NBM.

The pathogenesis of cold restraint-induced gastric lesions (erosions and petechiae) are still not completely understood and involves various factors. In this sense, factors such as degree of hypothermia, increased gastric muscular contractility, diminished mucosal blood flow, mast cell degranulation, activated polymorphonuclear leukocytes, release of biogenic amines and lipid peroxidation have been suggested as variables highly related to the production of gastric damage by cold restraint stress (18,19,20,21,22,23,24). However, gastric acidity has a minor role in cold restraint induced lesions (23,25). On the other hand, some of our previous studies suggested that the pathogenesis of cold restraint-induced gastric lesions and petechiae may be different. Namely, vagal overactivity is important only for the production of gastric erosions while histamine release is highly involved in the production of both type of gastric lesions (erosions and petechiae) in cold restraint stressed rats (25,26,27). In addition, it was found that restraint stress only produced gastric petechiae, being the model vagal overactivity only found in the first 60 min of stress. However, in cold restraint stress, where the vagal overactivity was prolonged, both petechiae and severe haemorrhagic ulcers were found (28).

On the other hand, it has been reported that cold restraint stress induced a significant increasing of brain calcium levels, which resulted on enhanced dopamine synthesis and high levels of gastric ulcer formation (29). Several studies were conducted with the aim to investigate the effect of a phenylalkylamine calcium channel blocker, verapamil, on gastric function as well as on the development of gastric mucosal lesions in cold restraint model. It was shown that verapamil pretreatment can prevent cold restraint-induced gastric stress ulcer (9,30).

Based on the knowledge of the mechanisms involved in gastric stress ulcer production, several gastric functions were the subject of investigations in previously verapamil-treated rats exposed to cold restraint. Verapamil decreased total and free gastric acidity without changing

the gastric secretory volume (9, 31, 32, 33). However, it was found that, in rats, cold restraint produced a significant decrease in gastric acid production (25,34,35,36,37). Besides that, some of the doses of verapamil which did not significantly influence the acid output, also had antiulcer effect (31). These results imply that the antisecretory action of verapamil may not account for its antiulcer effect in a cold restraint model for stress ulcer production.

It has been shown that in rats cold-water immersion restraint stress (38,39) as well as cold restraint stress (21) are associated with increased contractility of the stomach. Moreover, Garrick et al. (39) reported that rats in which such contractions lasted more than 1 hr, developed gastric mucosal lesions, whereas those animals in which the contractions lasted less than or equal to 1 hr, had no lesions. This implies that gastric hypermotility plays one of the main roles in stress ulceration. Other studies have indicated that the frequency of gastric contractions was significantly increased during the first hour but, during the second hour of cold restraint gastric motility significantly decreased (40,41). On the other hand, Koo et al. (40) reported that, although the gastric tone initially falls, it rises during the second hour of cold restraint stress. Besides that, verapamil pretreatment suppressed the frequency and amplitude of gastric contractions (21,41). Based on their findings, Koo et al. (40,41) concluded that: i) gastric hypermotility only contributes partly to the ulceration process in cold restraint and ii) the stomach smooth muscle-relaxing action of verapamil is not primarly involved in their antiulcer effect.

Cold restraint stress significantly depletes stomach wall mucus, while verapamil significantly prevents stress-induced mucus depletion, thus implying that the antiulcer effect of verapamil could partly be due to the preservation of gastric mucus (42). Moreover, the possible antiulcer effect of verapamil could be related to decreased amine release from the mast cells, since cold restraint stress significantly induce mast cell degranulation (21).

The antiulcer activity of verapamil could be also related to the prevention of the increase in gastric lipid peroxidation due to stress (12,43).

Besides that, the protective effect of verapamil against cold restraint induced stress ulcer, could be mediated by the prostaglandin E_2 (PGE_2). Namely, PGE_2 is an important factor in the maintenance of the integrity of the gastric mucosa whereas cold restraint induced a significant decrease of the level of PGE_2 in the gastric mucosa (44). On the other hand, verapamil significantly increase the gastric mucosa level of PGE_2 in animals exposed to cold

restraint (44).

The antiulcer effect of verapamil could be also related to increase of the body temperature since it was found that verapamil increase the core body temperature in NBM-lesioned rats (45).

Based on the knowledge that the pathogenesis of cold restraint-induced gastric petechiae and erosions could be partly different, the results of the present study do not show an ulcer index as a representative parameter of the gastric pathology. So that, instead of the ulcer index, the number of petechiae and erosions as well as the length of the erosions were presented. The present study indicated that verapamil, in doses of 5.0 and 10.0 mg/kg significantly reduced the number and length of erosions in control animals. However, the same doses of verapamil, in NBM-lesioned rats, significantly diminished the number of petechiae but did not significantly reduce the number or length of erosions. Since verapamil expressed different effects in the prevention of the two types of gastric lesions, in control and NBM-lesioned rats, it can be suggested that the gastric ulcer pathogenic process differs following lesions of a cholinergic brain structure such as NBM. Although, verapamil has been proposed for the treatment of ulcer patients, the present findings suggest that, since verapamil abolish only the development of one type of gastric lesions, this drug can't be used as a primarly antiulcer drug. The differences obtained between control and NBM-lesioned rats generates more questions about the origin of these two types of cold restraint induced gastric lesions and since the NBM-lesioned rat can be considered an animal model of AD, these data also raise questions about the adequate antiulcer treatment in Alzheimer's patients.

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