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THE ROLE OF THE NEUROTRANSMITTERS ACETYLCHOLINE AND NORADRENALINE IN THE PATHOGENESIS OF STRESS ULCERS

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Abstract—1. Adrenergic and cholinergic mechanisms seem to be involved in the pathogenesis of stress ulcers.

2. In this study, gastric ulcers were induced in rats by immobilization and cold. Prior intraperitoneal administration of both anticholinergic (atropine) as well as α-blocking medication (phenoxybenzamine) produced a very significant decrease in stress ulcers.

3. Additionally, using the technique of continuous intravenous perfusion in rats, acetylcholine was shown to have a gastric ulcerogenic effect, in contrast to noradrenaline.

4. It is concluded that acetylcholine is the peripheral mediator in stress ulcers, while noradrenaline intervenes at the encephalic level in stress ulcer pathogenesis.

INTRODUCTION

Selye (1936a) introduced the term "stress" to designate the psycho-physical aggressions which a living being can suffer, and which demand a general reaction in order to face them. In this reaction or general adaptation syndrome, a series of physiological changes arises, their pathogenesis being produced by a cortico-adrenal hyperfunction through the hypothalamus–hypophysis-adrenal axis, in addition to a sympathetic predominance which Cannon (1929) had already described in fight–flight reactions. Shortly after this, Selye (1936b) described the appearance of acute ulcers in the gastric mucosa of rats submitted to stress. He further observed that the immobilization of the rat constituted a stress for the animal, as it reproduced the manifestations of the adaptation syndrome. Since then, immobilization of a laboratory animal has been a widely-used experimental model for the study of stress manifestations (Paré *et al.*, 1986). The study of stress is fundamental in surgery and in psychosomatic medicine.

Hume and Egdahl (1959) highlighted the importance of the central nervous system in the genesis of stress, demonstrating that the responses to stress could be lessened when the central nervous system was eliminated from the reflex arc. It seems to be that the afferences of psycho-physical stress are projected on the central nucleus of the amygdala (CEA), which induces the peripheral neuroendocrine responses to stress by means of its amygdalafugal projections on the hypothalamus (Luparello, 1967; Henke, 1980; Henke, 1988; Ray *et al.*, 1988; Fernández de la Gándara *et al.*, 1993). Both the mesolimbic dopaminergic system and the GABA have protective or modulating effects on stress (Dasgupta *et al.*, 1967; File *et al.*, 1981; Hernández *et al.*, 1984; Hernández *et al.*, 1986; Ray *et al.*, 1988; Sullivan *et al.*, 1989).

In the pathogenesis of gastric ulcers due to stress, central factors (which are common throughout the syndrome) and peripheral factors intervene. In peripheral pathogenesis of these ulcers, the definitive factor seems to be a mucosal ischemia (Watanabe, 1966; Goldman *et al.*, 1968; Dai *et al.*, 1974), which could be produced by a vagal hyperactivity through a non-hypersecretory mechanism (Dai *et al.*, 1975). The vagal action also liberates histamine from the mastocytes, which acts on the H1 and H2 receptors, thus inducing a vasodilation which would in turn produce ulcers on ischemic mucosa (Cho *et al.*, 1979). The inhibition of prostaglandin synthesis could contribute to the ulcerogenic effect of the vagal action (Robert, 1979; Foschi *et al.*, 1986; Levine *et al.*, 1987). Vagal hyperactivity in stress might be produced by the stimulation of the dorsal nucleus of the vagus through amygdalin and hypothalamic afferences (Okumura *et al.*, 1989).

There are authors who think that the mucosal ischemia in stress could have a distinct origin, such as the α-adrenergic effects of noradrenaline (Djahanguiri *et al.*, 1968), among which splanchnic vasoconstriction is cited.

In previous studies, the authors have demonstrated experimentally the beneficial action of various tricyclic antidepressants on stress ulcers, possibly through their anticholinergic effects and central and peripheral α-blocking effects (Fernández de la Gándara *et al.*, 1993).

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From the previous work, it can be deduced that cholinergic and adrenergic mechanisms seem to be involved in the central or peripheral pathogenesis of stress ulcers. The objective of this study is to analyze pathogenesis of stress ulcers more deeply, and to see what role acetylcholine and noradrenaline play in it, at a central as well as a peripheral level.

MATERIALS AND METHODS

The laboratory animals used in this study were female rats of the Wistar breed, with an approximate weight of 200 g, which were left fasting (water free) for 24 hr before commencement of the experiment, in elevated metallic cages to prevent coprophagy.

1st Design. Stress ulcers

As stimuli to produce stress in the animals, immobilization and cold were chosen (Sennay *et al.*, 1967); the rats were thus introduced into special individual cages for restraint, and left in a cold chamber at 4°C for 7 hr. Following this period, the stomachs of the animals were removed, and their mucosa were examined for ulcers in a similar method to that utilized in previous studies (Fernández de la Gándara *et al.*, 1993). The sum of the lengths of the ulcers in each animal constituted the accumulative longitude of the ulcers.

Four groups of nine rats each were established, except for the sham group, which included 16 specimens. Group 1 was the control group, which received no stress-induction factors. Group 2 constituted the sham group, which was submitted to stress without receiving any type of pharmaceutical treatment. The remaining groups received, just before their immobilization, the following pharmaceutical products by intraperitoneal injection: Group 3, Phenoxybenzamine (Dibenzyline) 1 mg/kg of weight; and Group 4, Atropine (Palex) 1 mg/kg of weight.

The dose used was established taking into consideration the dose with α-blocking and anticholinergic effects of these medications on the rat (Barnes *et al.*, 1973).

2nd Design. Intravenous perfusion of neurotransmitters

The technique of continuous intravenous perfusion in unrestrained rats was originally created for experiments on parenteral nutrition (Steiger *et al.*, 1972; Giner *et al.*, 1989). On the one hand, this technique requires microscopic venotomy of the jugular vein of the rat, and on the other, it requires the use of a fine catheter covered with a flexible metallic protector to prevent the animal from gnawing the catheter. This is joined to a continuous perfusion pump of a volumetric type through a swivel or rotating connection of the catheter. The function of the swivel is to permit free turning of the animal, avoiding its immobilization as well as strangulation of the catheter. In our design, the animal was perfused through the swivel by a perfusion pump with a $\frac{1}{3}$ glucosaline solution, at a rhythm of 1 ml/hr. This solution, apart from being iso-osmotic, covers the basic hydrosaline needs of the animal and supplies the caloric minimum which prevents ketosis.

After 7 hr, the rat stomachs were extracted and examined for ulcers, following the same technique as described for the 1st design.

We established four groups of nine rats each. Group 1 constituted the sham group, to which only a clean glucosaline fluid was administered. The other groups received a continuous intravenous perfusion of the following compounds: Group 2, noradrenaline bitartrate (Sigma) 0.01 mg/kg of weight and minute, equivalent to 0.005 mg of base noradrenaline; Group 3, chlorhydrate of acetylcholine (Merck), 0.1 mg/kg of weight and minute; and Group 4, noradrenaline and acetylcholine, at the same dose.

The dose of noradrenaline was established bearing in mind the dose with α-adrenergetic effects of noradrenaline in bolus i.v. in the rat, and its half-life (Barnes *et al.*, 1972). The same procedure was followed for the acetylcholine.

Evaluation of results

The differences in the gastric lesions produced in the four groups of rats were analyzed by the comparison of means for independent data (Student's *t*-test with two tails).

RESULTS

1st Design. Stress ulcers

The results from the 1st Design are summarized in Table 1. The control group rats did not present any kind of lesions in their stomachs. The sham group rats, submitted to stress, presented acute ulcers or erosions in the gastric mucosa, distributed throughout the zone, respecting fundus, antrum and duodenum. The mean accumulative longitude (MAL) of the lesions in this group was 4.99 mm, with a standard error of the mean (SEM) of 1.20. In the group pre-treated with phenoxybenzamine prior to stress administration, a very significant statistical decrease in the MAL of the lesions was produced with respect to the sham group ($P < 0.011$), with a MAL of 1.40 and a SEM of 0.38. In the group pretreated with atropine, a decrease ($P < 0.003$) in the MAL of the ulcers was produced, with a a MAL of

Table 1. Effect of different medications on stress ulcers

Group	MAL	α	*n*
Control	0		9
Stress	4.99 ± 1.20		16
Stress + phenoxybenzamine	1.40 ± 0.38	$P < 0.011$	9
Stress + atropine	0.60 ± 0.44	$P < 0.003$	9

MAL: Mean of the accumulative longitude of the ulcers in mm ± SEM.
α: Probability of committing a type-1 error in comparing the MAL of each group with the stress group (Student's *t*-test with two tails).
n: Number of rats in each group.

Table 2. Ulcerogenic effect of continuous intravenous perfusion of different neurotransmitters.

Group	MAL	α	n
Perfusion	0.59 ± 0.27		9
Stress	4.99 ± 1.20	$P < 0.002$*	16
Perfusion	0.59 ± 0.27		9
Noradrenaline	0.30 ± 0.66	$P < 0.42$*	9
Acetylcholine	5.07 ± 1.40	$P < 0.014$*	9
Acetylcholine	5.07 ± 1.40		9
Acetylcholine + Noradrenaline	5.83 ± 2.04	$P < 0.761\Phi$	9
Acetylcholine	5.07 ± 1.40		9
Stress	4.99 ± 1.20	$P < 0.97\ \Phi$	16

MAL: Mean of the accumulative longitude of the ulcers in mm $\pm$ SEM.
α: Probability of committing type-1 error in comparing the MAL of each group with the perfusion group (*) or the acetylcholine group (Φ) (Student's t-test with two tails).
n: Number of rats in each group.

0.060 ± 0.44 mm., which was statistically significant ($P < 0.003$).

2nd Design. Intravenous perfusion of neurotransmitters

The results of the 2nd Design are presented in Table 2. In the group of unrestrained rats submitted to continuous intravenous perfusion with clean glucosaline solution, it can be seen that there were hardly any stomach ulcers produced, with a MAL of 0.59 mm and a SEM of 0.27. Comparing, statistically, the MAL of the ulcers from this group with those of the group submitted to stress, it can be seen that the perfusion technique produced ulcers whose MAL was much smaller than those of the stress group, with differences which were very significant statistically ($P < 0.002$). The perfusion of acetylcholine produced ulcers whose MAL was of 5.07 mm with a SEM of 1.40, statistically significant differences existing ($P < 0.014$) in comparison with the clean glucosaline perfusion group. In contrast, the perfusion of noradrenaline did not produce ulcers with statistically-significant differences in their MAL compared with those of the clean glucosaline perfusion group ($P < 0.66$). As no significant results were obtained with noradrenaline at the dose indicated, the experiments were repeated with a dose of 0.03 mg/kg of weight and minute of noradrenaline bitartrate, resulting in the death of most of the animals. The simultaneous perfusion of acetylcholine and noradrenaline produced ulcers whose MAL were of 5.83 with a SEM of 2.04. Comparing the MAL of the ulcers of the group to which only acetylcholine was perfused with the MAL of the ulcers of the group to which acetylcholine and noradrenaline together were perfused, it can be seen that there are no statistically-significant differences between the two groups ($P < 0.761$). Finally, comparing the differences existing between the MAL of the ulcers produced by stress with the MAL of the ulcers produced by the perfusion of acetylcholine, it can be noted that no significant differences exist from a statistical point of view ($P < 0.97$). Furthermore, we can assert that they are equal.

DISCUSSION

The stress induced in our rats by immobilization and cold produced acute ulcers in their stomachs, whose mean accumulative longitude is similar to that indicated by other authors (Cho *et al.*, 1979; Fernández de la Gándara *et al.*, 1993).

In the group pretreated with anticholinergic drugs, a decrease in the MAL of the ulcers was produced, which was very significant statistically ($P < 0.003$) with respect to the control group. These results draw attention to the importance of acetylcholine and the vagus in the pathogeny of stress ulcers, as numerous authors have already pointed out, given that vagal stimulation produces ulcers (Cho *et al.*, 1976), and both atropine as well as vagotomy prevent the formation of stress ulcers (Hume *et al.*, 1959; Watanabe, 1966; Goldman *et al.*, 1968; Dai *et al.*, 1974; Foschi *et al.*, 1986). The mechanism by which atropine or vagotomy prevents stress ulcer formation does not appear to be antisecretory, considering that gastric secretion decreases in stress (Dai *et al.*, 1974). It seems more likely that the vagus induces stress ulcers by means of a vascular mechanism producing an ischemia of the gastric mucosa (Watanabe, 1966; Goldman *et al.*, 1968; Dai *et al.*, 1975; Cho *et al.*, 1979), perhaps through muscular contractions of the stomach wall, on which the vasodilation produced by the histamine liberated by the vagal stimulus would act, producing the ulcers.

In the group pretreated with α-blocking drugs (phenoxybenzamine), a highly statistically-significant decrease in the MAL of the lesions was produced ($P < 0.011$). This agrees with the fact that in states of stress, a dominance of the sympathetic system exists. Furthermore, this would also suggest that it is the noradrenaline itself which produces the stress ulcers through its α effects. It has been demonstrated that, in stress ulcers, there is a decrease in the blood flow in the gastric mucosa (Goldman *et al.*, 1968), of debatable pathogenesis, but which appears to contribute to the formation of ulcers. Likewise, it has been demonstrated that in states of shock, acute ulcers of the gastric mucosa are produced, due to the

damage resulting from ischemia, and more specifically from posterior reperfusion, on the gastric mucosa (Horjola *et al.*, 1966). Given that splanchnic vasoconstriction is found among α-adrenergic effects, it is possible that noradrenaline generates stress ulcers through the damage produced in the gastric mucosa by the ischemia originated by the splanchnic vasoconstriction resulting from the α-adrenergic effects. In addition, among such effects, one also finds a decrease in digestive secretions, a circumstance which would explain the curious fact that gastric secretion is lowered in stress ulcers (Dai *et al.*, 1974). This would confirm the findings of Djahanguiri (1968), in which α-blocking agents (phentolamine) prevent stress ulcers.

On the other hand, it has been demonstrated that the peripheral afferences that carry the stress to the encephalon are carried by the vagus to the solitary tract in the brain stem (Hume *et al.*, 1959). Based on this, it has been further demonstrated that afferences reach the CEA, and that the principal neurotransmitter in this route is noradrenaline (Zardetto-Smith *et al.*, 1990). Therefore, phenoxybenzamine (which crosses the hematoencephalic barrier) (Goodman Gilman *et al.*, 1990) could prevent the formation of stress ulcers by means of its central effects, blocking stress reception by the CEA.

From all of the foregoing, it seems possible to deduce that both acetylcholine as well as noradrenaline play an important role in the pathogenesis of stress ulcers, both at a central level as well as a peripheral level. In order to clarify the exact role these neurotransmitters play at the peripheral level in ulcerogenesis, in this study the authors administered the substances in question intravenously with continuous perfusion to unrestrained rats (not immobilized), due in part to the pharmaco-kinetic effect of these neurotransmitters (Goodman Gilman *et al.*, 1990), and due in part to the fact that immobilization itself of the animal produces stress ulcers.

In Table 2 it can be seen that the technique of continuous intravenous perfusion barely produced ulcers in the stomachs of unrestrained rats. That is to say, this technique produces acute ulcers due to the stress implied, but both the ulcers as well as the stress are minimal, as shown by the statistical comparison of the MAL of the ulcers in this group with that of the group submitted to stress through immobilization and cold. The MAL of this group was much smaller, with very significant statistical differences ($P < 0.002$). This technique can therefore be used to see the possible peripheral ulcerogenic effect of different neurotransmitters.

The intravenous perfusion of acetylcholine produced gastric ulcers with statistically-significant differences ($P < 0.014$) with respect to the group in which only a clean glucosaline solution was perfused, thus indicating that acetylcholine has a peripheral ulcerogenic effect. On the other hand, there are no significant differences from a statistical point of view ($P < 0.97$) between the MAL of ulcers produced by stress and the MAL of the ulcers produced by acetylcholine perfusion. On the contrary, it seems to affirm that, statistically, they are equal. Furthermore, in both groups, acute ulcers were produced exclusively in the mucosa of the body of the stomach, respecting fundus, antrum and duodenum. In addition, atropine has previously been shown to prevent the formation of stress ulcers. Therefore, it can be stated that acetylcholine not only has a peripheral ulcerogenic effect, but is also quite probably the peripheral mediator in stress ulcers. This agrees with the already demonstrated fact (Watanabe, 1966; Goldman *et al.*, 1968; Cho *et al.*, 1979; Foschi *et al.*, 1986) that atropine as well as vagotomy block stress ulcers, and with the fact that both electrical stimulation of the vagus (Cho *et al.*, 1976) and stimulation of the dorsal nucleus of the vagus by injection of Kainic acid (Okumura *et al.*, 1989) are capable of producing severe gastric ulcers.

In our study, continuous intravenous perfusion of noradrenaline did not produce ulcers with statistically-significant differences in their MAL as compared to those of the clean glucosaline perfusion group ($P < 0.66$). Upon increasing the dose, the animals died; this suggests that noradrenaline lacks any peripheral ulcerogenic effect. In addition, the simultaneous perfusion of acetylcholine and noradrenaline produced ulcers whose MALs were not significantly different ($P < 0.76$) from that of those produced by acetylcholine perfusion. This means that noradrenalinee, in addition to lacking any peripheral ulcerogenic effect, does not potentiate the ulcerogenic effect of acetylcholine.

However, we have seen how α-blockers (phenoxybenzamine) possess a protective action against stress ulcers. Likewise, in previous studies, the authors have demonstrated that the various actions of different anti-depressants on stress ulcers can be explained, in part, by an adrenergic mechanism (Fernández de la Gándara *et al.*, 1933). These phenomena can be explained by the fact that both tricyclic antidepressants as well as phenoxybenzamine cross the hematoencephalic barrier. However, as this is not true in the case of noradrenaline (Goodman Gilman *et al.*, 1990), the adrenergic effects of anti-depressants and α-blockers on stress ulcers can occur at the central encephalic level. Specifically, they could act on a noradrenergic path which reaches the CEA through the solitary tract of the brainstem (Zardetto-Smith *et al.*, 1990), which leads the stress afferences. Thus, the results suggest that noradrenaline intervenes in the pathogenesis of stress ulcers at the central level, while it lacks peripheral ulcerogenic effects.

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