11. I. S. Hochman and B. H. Bulkley, Am. J. Cardiol., 50, 83 (1982).
12. G. I. Jesmok, D. C. Warltier, G. J. Gross, et al., Basic Res. Cardiol., 73, 559 (1978).
13. T. Peter and M. K. Heng, Circulation, 57, 534 (1978).
14. M. M. Rasmussen, K. A. Reimer, R. A. Kloner, et al., Circulation, 56, 794 (1977).
15. R. D. Reynolds, W. E. Burneister, R. J. Gorczynnski, et al., Cardiovasc. Res., 15, 411 (1981).
16. A. J. Roberts and P. R. Cipriano, Circulation, 57, 35 (1978).
17. W. E. Shell and B. E. Sobel, Am. J. Cardiol., 31, 157 (1973).

ROLE OF DOPAMINE RECEPTORS IN THE MECHANISM OF STRESS INJURIES OF THE STOMACH

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The writers showed previously that the protective effect of metoclopramide (MC) against stress injuries of the gastric mucosa (GM) in rats is not due to its ability to stimulate the motor-evacuatory function of the stomach, for it is exhibited against a background of atropine and in rats with a ligature applied to the pyloric sphincter [2]. Attention is directed to the specificity of the gastroprotective action of MC, which gave the best protection of GM against massive hemorrhages. In the light of modern views on the mechanism of action of MC, to which the properties of a selective blocker of dopamine receptors (DR) are ascribed [7, 8, 10], it has been suggested that one cause of stress injury to GM in rats is excitation of DR. Since MC blocks DR both centrally and peripherally, the site of dopamine excitation has not been identified.

The aim of the present investigation was to test this hypothesis and to clarify the role of excitation of DR in the CNS and at the periphery in ulcerogenesis.

EXPERIMENTAL METHOD

The investigation was carried out on 300 albino rats of both sexes weighing 150-200 g, which were deprived of food for 24 h before the experiment, but allowed water ad lib. Two models of combined immobilization ulcer were used: immobilization + generalized electrization (GE) [3] and immobilization + the action of "social stress" (SS) [1], i.e., keeping the immobilized animals in a colony of unrestrained rats. For pharmacologic stimulation of DR the rats were given an intraperitoneal injection 30 min before exposure to stress of the dopamine agonist apomorphine, in doses of 2-17 mg/kg, dopamine in doses of 3.125-50.0 mg/kg, and the dopamine precursor L-dopa in doses of 30-240 mg/kg. Some experiments were carried out on vagotomized animals; the rats were used in the experiments 7 days after bilateral subdiaphragmatic vagotomy. The animals were killed 24 h after exposure to stress, the stomach was removed, and by means of a transillumination gastroscope and magnifying glass, the presence of ulcers and hemorrhagic lesions was determined on the surface of GM; the lesions were differentiated into ulcers, erosions, and massive hemorrhages, in accordance with the principle adopted previously [1]. The number of lesions in each animal and the number of animals with stomach lesions were counted.

EXPERIMENTAL RESULTS

Injection of L-dopa and apomorphine into the rats in doses of 60 and 16 mg/kg respectively without exposure to stress had no effect on the state of GM (Table 1). Apomorphine,

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TABLE 1. Effect of L-Dopa, Apomorphine and Dopamine on Experimental Ulcer Formation under Conditions of Social Stress (number of lesions per animal)

Type of lesion	Control (n = 43)	L-dopa, mg/kg				
		60 without stress (n = 12)	30 (n = 11)	60 (n = 24)	120 (n = 24)	240 (n = 18)
Ulcer	0,42±0,40 (40,0%)	0	0,09±0,09 (9,1%)	0	0	0,1±0,07 (11,1%)
Erosion	2,82±0,61 (92,2%)	0,91±0,35 (41,7%)	0,82±0,29 (54,5%)	0,62±0,21 (29,2%)	1,29±0,82 (54,2%)	0,55±0,22 (33,3%)
Massive hemorrhage	2,11±0,56 (50,0%)	0,33±0,25 (16,6%)	1,82±0,71 (54,5%)	4,46±0,62 (83,3%)	1,96±0,68 (33,3%)	2,92±0,73 (66,6%)

Apomorphine, mg/kg		Control (n = 6)	Dopamine, mg/kg				
16 without stress (n = 14)	16 (n = 6)		3,125 (n = 7)	6,25 (n = 12)	12,5 (n = 12)	25,0 (n = 6)	50,0 (n = 12)
0	0	0	0	0	0	0	0
1,07±0,37 (50,0%)	0,67±0,33 (50,0%)	1,17±0,32 (83,3%)	0,57±0,31 (42,8%)	1,75±0,5 (58,3%)	0,75±0,23 (50,0%)	0,17±0,01 (16,6%)	1,0±0,44 (50,0%)
0,07±0,07 (7,1%)	3,5±0,99 (83,3%)	2,52±0,61 (83,3%)	3,0±1,33 (71,4%)	3,17±0,42 (100%)	3,42±0,62 (91,7%)	0,17±0,02 (16,6%)	0,83±0,4 (33,3%)

Legend. Here and in Tables 2 and 3, percentage of animals with lesions shown in parentheses.

TABLE 2. Effect of Apomorphine and L-Dopa on Experimental Ulcer Formation during General Electrization (number of lesions per animal)

Type of lesion	Control (n = 15)	Apomorphine, mg/kg				L-dopa, mg/kg			
		2 (n = 10)	4 (n = 12)	8 (n = 12)	16 (n = 11)	30 (n = 11)	60 (n = 12)	120 (n = 12)	240 (n = 10)
Ulcer	0,50±0,29 (33,0%)	0,3±0,2 (20,0%)	0,58±0,33 (25,0%)	0,33±0,14 (33,3%)	0	0,09±0,09 (9,1%)	0,33±0,19 (25,0%)	1,16±0,39 (50,0%)	1,08±0,15 (41,7%)
Erosion	3,8±0,88 (67,0%)	3,2±0,35 (70,0%)	3,0±0,9 (75,0%)	3,3±0,86 (75,0%)	2,9±0,46 (100%)	4,72±0,86 (90,9%)	2,17±0,6 (83,3%)	2,33±0,81 (66,7%)	1,42±0,43 (75,0%)
Massive hemorrhage	1,5±0,75 (58,3%)	0	0,66±0,00 (8,3%)	0	0,18±0,12 (18,2%)	1,45±0,3 (36,4%)	0,83±0,37 (41,7%)	0,5±0,15 (25,0%)	0,75±0,3 (41,7%)

injected during GE, weakened the intensity of stress damage to GM (Table 2), in agreement with the results of other investigations [13]. A different picture was observed when the drug was injected against a background of SS (Table 1): apomorphine, which considerably inhibited the appearance of ulcers and erosions, increased by 50% the number of hemorrhagic lesions in the stomach.

The effects observed after injection of L-dopa into rats exposed to SS depended on the dose of the drug given (Table 1). Whereas in a dose of 30 mg/kg L-dopa had a distinctly protective action against ulcers and erosions of GM and did not affect the vascular effects, under the influence of L-dopa in a dose of 60 mg/kg, against the background of still further weakening of ulcer and erosion formation, there was a twofold increase in the number of massive hemorrhages in GM and an increase of almost half in the number of rats with such lesions. This effect of L-dopa was not exhibited in vagotomized rats (Table 3). With a further increase in the dose of L-dopa (to 240 mg/kg) its protective effect against gastric ulcers and erosions was reduced and, at the same time, the ability of L-dopa to stimulate the formation of massive hemorrhages was sharply reduced.

A different picture was observed when L-dopa was injected into rats before exposure to GE (Table 2). In these experiments large doses of L-dopa (120 and 240 mg/kg) caused a twofold increase in ulcer formation but reduced the incidence of massive hemorrhages in each rat, although the number of rats with gastric lesions showed little change. On the one hand, therefore, the reciprocity of action of L-dopa on the formation of ulcers and massive hemor-

TABLE 3. Effect of Vagotomy, Metoclopramide, and Sulpiride on Ulcerogenic Effects of L-Dopa under Social Stress Conditions (number of lesions per animal)

Type of lesion	Control (n = 43)	L-dopa, 60 mg/kg (n = 24)	Vagotomy + L-dopa 60 mg/kg (n = 13)	Metoclopramide 0.04 mg/kg + L-dopa 60 mg/kg (n = 17)	Sulpiride 10 mg/kg + L-dopa 60 mg/kg (n = 17)
Ulcer	0,42±0,40 (40,0%)	0	0	0	0
Erosion	2,8±0,61 (92,0%)	0,6±0,21 (29,2%)	1,84±0,33 (84,6%)	0,47±0,15 (41,2%)	0,47±0,06 (41,2%)
Massive hemorrhage	2,1±0,56 (50,0%)	4,46±0,62 (83,3%)	0,31±0,21 (15,4%)	1,29±0,5 (35,3%)	1,0±0,32 (41,2%)

rhages in GM was confirmed (potentiation of ulcerogenesis was accompanied by a decrease in the formation of massive hemorrhages and vice versa), on the other hand the effect of L-dopa against the background of SS was opposite in direction to during the action of GE. These effects, despite their opposite character, are the result of the action of L-dopa, i.e., of excitation of DR, for they are abolished equally both by MC and by sulpiride (Table 3).

Unlike apomorphine and L-dopa, dopamine given under conditions of SS had only a protective action on GM, which became particularly marked when dopamine was given in a dose of 25 mg/kg (Table 1). In this dose dopamine reduced the number of erosions more than sixfold and the number of massive hemorrhages by almost 15 times [1]. A further increase in the dose of DA was accompanied by a decrease in its protective action on GM.

Analysis of the results and of data in the literature support the hypothesis expressed by the writers previously on the role of excitation of DR in stress ulcerogenesis and clarifies the nature of these DR and their localization. The fact that injection of L-dopa and apomorphine do not cause statistically significant lesions of GM (Table 1) is evidence that excitation of DR does not itself facilitate injury to GM or, in particular, the appearance of massive hemorrhages in it, but acts as a potentiating element of the state of stress. The central nature of this effect is demonstrated by the absence of any hemorrhagic action of L-dopa after bilateral vagotomy, and also the fact that like MC, GM also was protected by sulpiride, a well-known blocker of dopamine receptors in the CNS (Table 3). The weaker intensity of the effects of AM compared with those of L-dopa on stress-induced gastric lesions is evidence of involvement of D_2 DR to a greater degree than of D_1 DR in this effect [11].

It must be pointed out that besides its ability to promote the development of massive hemorrhages through the potentiation of stress, stimulation of DR under certain conditions has a protective action on the stomach in relation to lesions and erosions of GM, in agreement with data in the literature [4, 9]. This effect is exhibited during the action of L-dopa and apomorphine, but it was particularly marked in experiments in which dopamine was used. Since dopamine, unlike L-dopa and apomorphine, does not penetrate into the CNS [5], it can be postulated that its antiulcerogenic action is exhibited through excitation of DR at the periphery. In other words, if excitation of DR in the CNS potentiates the state of stress in rats and increases the vulnerability of GM to injury, particularly on account of massive hemorrhages, stimulation of peripheral DR has the opposite action. This is in agreement with the effects of stimulation of DR at the periphery — inhibition of gastric secretion and movements [6, 9, 12]. The possible role of conversion products of dopamine, adrenalin, and noradrenalin in these processes likewise cannot be completely ruled out.

The protective effect of L-dopa on vascular lesions of GM in rats exposed to GE requires discussion, more especially because GE itself induces vascular lesions of the stomach more often than SS, and which are effectively abolished by MC [2]. GE, as a powerful stressor stimulus, evidently induces maximally effective excitation of DR in the CNS. Additional injection of L-dopa against this background can no longer intensify vascular damage to the stomach. Conversely, it causes desensitization of DR, which leads to weakening of the vascular and intensification of the ulcerative lesions of the stomach.

LITERATURE CITED

1. S. D. Groisman and T. G. Karevina, Abstract deposited at VINITI, No. 12, b/o 131 (1979).
2. S. D. Groisman and T. G. Karevina, Fiziol. Zh. Akad. Nauk Ukr. SSR, No. 3, 39 (1982).

3. O. N. Zabrodina, in: Abstracts of Proceedings of a Conference of Junior Scientists of the Institute of Experimental Medicine [in Russian], Leningrad (1964), p. 83.
4. I. S. Zavodskaya, E. V. Moreva, Yu. D. Isachenko, et al., Farmakol. Toksikol., No. 5, 595 (1972).
5. M. D. Mashkovskii, Therapeutic Substances [in Russian], Part 1, Moscow (1977), p. 152.
6. D. M. Berkowitz and R. W. McCallum, Clin. Pharmacol. Ther., 27, 414 (1980).
7. P. R. Blower, J. D. Flack, and M. M. Day, Br. J. Pharmacol., 61, 1248 (1977).
8. I. M. Donaldson, P. Jenner, C. D. Marsden, et al., Br. J. Pharmacol., 56, 373P (1976).
9. J. Hano, J. Bugajski, and L. Danek, Pol. J. Pharmacol., 29, 629 (1977).
10. P. Jenner, C. D. Marsden, and E. Peringer, Br. J. Pharmacol., 54, 275P (1975).
11. B. Libet, Proc. Int. Union Physiol. Sci., 14, 180 (1980).
12. J. A. J. Schuurkes and J. M. Van Nueten, Scand. J. Gastroenterol., 16, Suppl. No. 67, 33 (1981).

ACTION OF KETOTIFEN ON SELECTIVE AND UNSELECTIVE HISTAMINE LIBERATION FROM HUMAN BASOPHILS

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The antiallergic drug ketotifen possesses a multiple pharmacologic action, which includes H_1-antihistamine activity [7, 10]. The writers showed previously that ketotifen selectively (noncytotoxically) releases histamine from mast cells [2], unlike other H_1-antihistamine agents, which have an unselective (cytotoxic) histamine-releasing action (HRA) on the target cells of allergy [3]. In doses possessing HRA, ketotifen inhibits histamine secretion from mast cells due to another selective activator of histamine secretion [6]. There is reason to suppose that differences exist in the mode of action of ketotifen on mast cells and basophils, for the inhibitory action of ketotifen on histamine secretion from basophils is exhibited in the absence of its HRA [15]. Recently published data showing that ketotifen has a cytotoxic HRA on basophils are in conflict with the information described above [11].

To test these contradictions an investigation was carried out to assess the action of ketotifen on HRA and to inhibit histamine release from human basophils caused by selective and unselective liberators.

EXPERIMENTAL METHOD

Blood was obtained from six clinically healthy donors (two men and four women) aged from 20 to 40 years. Tests were carried out twice on cells from each donor, from whom blood was taken (up to 40 ml) at intervals of between 3 and 7 days. Mononuclear cells, enriched with basophils (2-4% of basophils) were separated from the blood by centrifugation on a one-step Ficoll-Verografin density gradient (1.080 g/cm^3) [13]. The cells were incubated without and in the presence of the test substances for the necessary period of time at 37°C in 200 or 400 µl of buffer of the following composition (in mM): Tris-HCl (from Sigma) 25, pH 7.6; NaCl 120, KCl 5, $MgCl_2$ 1.15, $CaCl_2$ 0.6, glucose 5; human serum albumin 0.3 mg/ml. A buffer of the same composition but without Ca^{++} and Mg^{++} was used to isolate and wash the cells. The reaction was stopped by addition of 2 ml of cold buffer to the cells and placing the tubes on ice. Cells were sedimented by centrifugation at 2000g and 4°C for 10 min. The supernatant was discarded and the histamine content determined microspectrofluorometrically in the residual portions without preliminary extraction [13]. Ketotifen hydrogen fu-

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