STUDIES ON THE EXPERIMENTAL GASTROINTESTINAL ULCERS PRODUCED BY RESERPINE AND STRESS

3. EFFECTS OF MONOAMINES AND THEIR PRECURSORS

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Kido (1) has shown that even a small dose of reserpine could produce severe ulcers in the gastrointestinal tract of cats when combined with stress, such as hypothalamic stimulation and electrical shock through the grid on the floor of the cage. Hartry (2) and Sawrey and Sawrey (3) also reported that ulcers were produced by a small dose of reserpine and stress in rats.

The author (4,5) previously reported that ulcer formation by reserpine and stress had a close correlation with the decrease in monoamines, i.e. serotonin and norepinephrine, in the gastrointestinal tract, and that syrosingopine and tetrabenazine could produce similar ulcers when combined with grid shock. From these findings he speculated that the decrease in peripheral as well as central monoamines contributed important factors in ulcer formation by reserpine and grid shock. If it is true that the decrease in monoamines is the cause of ulcer formation by reserpine and grid shock, it has to be inhibited by a replenishment of monoamines or their precursors.

In the present study, the effects of MAO inhibitor, monoamines and their precursors on ulcer formation by reserpine and grid shock were investigated. Moreover, the effects of drugs which block the autonomic nerves were also investigated to confirm the observation of Kido (1) that in splanchnectomized and vagotomized cats ulcers were hardly produced by reserpine and grid shock.

METHODS

One hundred and twenty-two cats of both sexes weighing from 2.5 to 4.0 kg were used. All animals were deprived of food for 16 hours prior to the experiments. Reserpine was administered subcutaneously. Two hours later, cats were placed in individual cages equipped with grids on the floor and 5-seconds of electrical shock were given every 30 seconds for 4 hours to their body surfaces through the grids (abbreviated as grid shock). The intensity of grid shock was adjusted so that shocks always elicited invariable emotional responses.

At the end of grid shocks, cats were sacrificed by decapitation under pentobarbital anesthesia. The stomach adjoining 15 cm of duodenum was removed, cut along the greater curvature and photographed. Thereafter the tissue was fixed in 10% of formalin

and examined histologically by means of hematoxylin-eosin staining. The severity of ulcers produced was determined by the grading method which was described in a previous paper (5).

Insertion of cannulae: In some cats, chronic cannulae made of vinyl tubing were inserted in order to administer compounds such as 5-HTP, dopa, serotonin and norepinephrine. The animal was anesthetized by 30 mg/kg of sodium pentobarbital and the tip of the cannulae was inserted into the jugular vein about 7 cm long. The other tip was led under the skin to the head and fixed to the skull by dental cement. The inside of the cannulae was filled with saline. After a postoperative period of 4 to 5 days, animals were used for experiments. Administration of saline in this method caused no changes in the consciousness level and behavior of the animals.

Administration of monoamines and their precursors: Aqueous solution of norepinephrine hydrochloride, serotonin creatinine sulfate, L-dopa and 5-hydroxy-DL-tryptophane were administered through the cannulae described above. Each compound except norepinephrine was given every 30 minutes one hour after reserpine injection for 10 times and norepinephrine was given every 15 minutes for 20 times. L-Dopa was dissolved in dilute hydrochloric acid with gentle warming, a small amount of polyethyleneglychol added and the solution neutralized with dilute NaOH. 5-HTP and serotonin creatinine sulfate were dissolved in distilled water with gentle warming. Norepinephrine solution was prepared from a commercial stock solution (Sankyo).

Administration of atropine, dibenamine and isocarboxazid: Atropine methylnitrate was administered intravenously at the same time as reserpine injection. Dibenamine hydrochloride was administered subcutaneously one hour before the beginning of grid shock. These drugs were dissolved in distilled water. Isocarboxazid, suspended in arabic gum solution, was administered subcutaneously one hour before reserpine injection.

RESULTS

1. *Effects of monoamines and their precursors*

a) *5-Hydroxy-DL-tryptophane and L-dopa*

All animals of the control group which were administered 0.1 mg/kg of reserpine and grid shock for 4 hours, two hours after reserpine, presented severe ulcers as shown in Fig. 1.

5-HTP administered intravenously every 30 minutes for 10 times in a dose of 5 mg/kg (50 mg/kg, in total dose) markedly inhibited ulcer formation by reserpine and grid shock. In this group no animal presented evident ulcer, though a few slight erosions and haemorrhages were observed in some animals (Table 1, Fig. 2).

Dopa administered by the same method and dose as 5-HTP showed almost similar inhibitory effect on ulcer formation by reserpine and grid shock. In this case two out of five animals presented evident but slight graded ulcers. The mean score of lesions of this group was slightly greater than that for the 5-HTP administered group (Table 1).

In the group in which dopa and 5-HTP were administered alternatively every 30 minutes, each in a dose of 5 mg/kg (each 25 mg/kg in total), four out of five animals pre-

FIG. 1. Control. Grid shocks were given for 4 hours, 2 hours after the administration of 0.1 mg/kg of reserpine.

TABLE 1. Effects of 5-HTP and dopa on ulcer formation by means of 0.1 mg/kg of reserpine and 4 hours grid shock.

Drugs*	Total dose (mg/kg)	No. of animals		Score of lesions			
		Used	With ulcer	Corp.	Pylor.	Duod.	Total
Control	—	5	5	0.6	3.8	2.2	6.6
L-Dopa	50	5	2	0.4	0	1.6	2.0
	10	5	5	0	2.4	1.8	4.2
DL-5HTP	50	5	0	0.6	0.4	0.8	1.8
	10	5	5	0.2	3.8	2.2	6.2
L-Dopa + DL-5HTP	25 + 25	5	4	0.2	0	2.0	2.2

* All animals were administered 0.1 mg/kg of reserpine and grid shock for 4 hours, 2 hours after reserpine. Animals in the control group were administered 1.5 ml of saline every 30 minutes one hour after reserpine and those in other groups were administered dopa or 5-HTP instead of saline.

FIG. 2. 5-HTP (50 mg/kg in total dose). Reserpine and grid shocks were given as control in Fig. 1.

sented small ulcers but the mean score of lesions of the total gastrointestinal tract of this group was not significantly different from those of the two groups mentioned above.

Sedateness and ptosis induced by 0.1 mg/kg of reserpine was counteracted by either 5 mg/kg of 5-HTP or the same dose of dopa. Some animals stood up from a lying posture and began to walk during the administration of these compounds. The effect of 5-HTP or dopa appeared 10 minutes after administration at the latest and were maintained for more than 30 minutes. Therefore, after the first administration of 5-HTP or dopa animals showed neither sedateness nor ptosis. In a few cases, even slight alertness and emotional excitation were observed.

When the dose of 5-HTP was decreased to 1 mg/kg/30 minutes (total dose, 10 mg/kg), ulcer formation by 0.1 mg/kg of reserpine and grid shock was merely decreased slightly (Fig. 3). As shown in Table 1, the mean score of lesions of this group was slightly less than that of the control group. The effect of dopa in the same dose was more marked than 5-HTP.

When the dose of reserpine was increased to 1.0 mg/kg, ulcers were more severe than for the case of 0.1 mg/kg and were produced in all animals (Fig. 4). These ulcers were reduced to about half by 5 mg/kg/30 minutes of dopa, but they were reduced only to a small extent by the same dose of 5-HTP (Table 2). The mean score of lesions of the group in which 5-HTP and dopa were administered alternatively every 30 minutes was almost middle between two groups in which 5-HTP or dopa were separately administered. The antagonistic effects of dopa and 5-HTP at this dose against sedation induced by 1 mg/kg of reserpine was not as marked as in the case of 0.1 mg/kg of reserpine with 5 mg/kg of 5-HTP or dopa.

FIG. 3. 5-HTP (10 mg/kg in total dose). Reserpine and grid shocks were given as control in Fig. 1.

FIG. 4. Lesions produced by 4 hours grid shock given 2 hours after the administration of 1.0 mg/kg of reserpine.

TABLE 2. Effects of 5-HTP and dopa on ulcer formation by means of 1.0 mg/kg of reserpine and 4 hours grid shock.

Drugs*	Total dose (mg/kg)	No. of animals		Score of lesions			
		Used	With ulcer	Corp.	Pylor.	Duod.	Total
Control	—	5	5	2.0	3.7	2.3	8.0
L-Dopa	50	5	5	1.0	2.0	1.2	4.2
DL-5HTP	50	5	5	0.9	2.5	2.5	5.9
L-Dopa + DL-5HTP	25+25	5	5	0.8	2.4	2.2	5.4

* Details as in Table 1.

Thus the inhibitory effects of 5-HTP and dopa on ulcer formation by reserpine and grid shock seemed to be in parallel with their antagonistic effects as observed by the behavior of the animals.

b) Serotonin and norepinephrine

Serotonin creatinine sulfate was administered every 30 minutes for 10 times one hour after the administration of 0.1 mg/kg of reserpine. The total dose of serotonin creatinine

TABLE 3. Effects of serotonin creatinine sulfate on ulcer formation by means of 0.1 mg/kg of reserpine and 4 hours grid shock.

Drugs*	Total dose (mg/kg)	No. of animals		Score of lesions			
		Used	With ulcer	Corp.	Pylor.	Duod.	Total
Control	—	5	5	0.6	3.8	2.2	6.6
Serotonin creatinine sulfate	1.0	5	4	1.2	3.4	1.2	5.8
	0.5	5	5	0.6	2.8	2.4	5.8
	0.1	5	4	0.6	2.8	1.0	4.4
	0.01	5	5	0.4	3.0	2.2	5.6

* Details as in Table 1.

sulfate and results obtained are listed in Table 3. The inhibitory effect on ulcer formation by reserpine and grid shock was observed in only one case, i.e. 0.1 mg/kg in total dose, though the effect was slight (Fig. 5). In other doses no inhibitory effect was observed. Moreover, in some animals administered 0.5 mg/kg (in total) of serotonin, ulcers more severe than that of the control group were obtained.

Norepinephrine was administered every 15 minutes for 20 times one hour after the administration of 0.1 mg/kg of reserpine. As shown in Table 4, slight inhibitory effect was observed in a dose of 0.1 mg/kg (in total), and in the cases of other doses used in this experiment ulcer formation by reserpine and grid shock was not modified. Neither serotonin nor norepinephrine could antagonize the sedation induced by reserpine.

FIG. 5. Serotonin (0.1 mg/kg in total dose). Reserpine and grid shocks were given as control in Fig. 1.

TABLE 4. Effects of norepinephrine on ulcer formation by means of 0.1 mg/kg of reserpine and 4 hours grid shock.

Drugs*	Total dose (mg/kg)	No. of animals		Score of lesions			
		Used	With ulcer	Corp.	Pylor.	Duod.	Total
Control	—	5	5	0.6	3.8	2.2	6.6
Norepinephrine	0.2	5	5	0.6	3.2	3.0	6.8
	0.1	5	5	0	2.4	2.8	5.2
	0.02	5	5	0.6	3.4	2.6	6.6

* Details as in Table 1.

2. Effect of MAO inhibitor

Ten and 20 mg/kg of isocarboxazid were administered subcutaneously one hour before reserpine injection and grid shocks were given two hours after reserpine. Ulcer formation by 0.1 mg/kg of reserpine and 4 hours grid shock was considerably inhibited by 10 mg/kg of isocarboxazid, but not by 20 mg/kg as shown in Table 5. Isocarboxazid administered alone in a dose of 20 mg/kg caused no pathological change.

TABLE 5. Effects of isocarboxazid on ulcer formation by means of 0.1 mg/kg of reserpine and 4 hours grid shock.

Drugs*	Dose (mg/kg)	No. of animals		Score of lesions			
		Used	With ulcer	Corp.	Pylor.	Duod.	Total
Control	—	5	5	0.6	3.5	2.9	7.0
Isocarboxazid	10	5	3	0	2.2	1.2	3.4
	20	5	5	1.0	2.0	3.0	6.0

* Grid shocks were given 2 hours after the subcutaneous administration of 0.1 mg/kg of reserpine for 4 hours. Isocarboxazid was administered subcutaneously 1 hours before the administration of reserpine.

TABLE 6. Effects of atropine methylnitrate and dibenamine-HCl on ulcer formation by 0.1 mg/kg of reserpine and 4 hours grid shock.

Drugs	Dose (mg/kg)	No. of animals		Score of lesions			
		Used	With ulcer	Corp.	Pylor.	Duod.	Total
Control*	—	5	5	0.8	3.7	1.9	6.4
Atropine methylnitrate	0.1	5	4	0	2.8	1.4	4.2
	0.5	5	1	0	1.0	0.8	1.8
	1.0	5	2	0	0.8	0.8	1.6
Control**	—	5	5	1.2	2.8	3.2	7.2
Dibenamine-HCl	10	7	6	0	3.0	1.6	4.6

* Grid shocks were given 2 hours after the subcutaneous administration of 0.1 mg/kg of reserpine for 4 hours. Atropine was administered intravenously at the same time as reserpine.

** Grid shocks were given 20 hours after the subcutaneous administration of 0.1 mg/kg of reserpine for 4 hours. Dibenamine was administered intravenously 1 hour before the beginning of grid shock.

3. Effects of dibenamine and atropine

a) Dibenamine

In preliminary experiments it was found that 10 mg/kg of dibenamine hydrochloride completely inhibited the pressor response to norepinephrine in anesthetized cats. Consequently, in this experiment, 10 mg/kg of the drug was used. As shown in Table 6, ulcer formation was slightly inhibited. This inhibitory effect was slightly less than that of surgical splanchnectomy by Kido (1).

b) Atropine

One tenth, 0.5 and 1.0 mg/kg of atropine methylnitrate were administered intravenously simultaneously with reserpine. Ulcer formation by reserpine and grid shock was markedly inhibited by atropine methylnitrate in doses exceeding 0.5 mg/kg (Fig. 6). Slight inhibition resulted from 0.1 mg/kg of atropine methylnitrate. Very similar effects as atropine methylnitrate were obtained by atropine sulfate, though the number of animals used was smaller. The behavior of animals was not modified by atropine methylnitrate or -sulfate.

FIG. 6. Atropine methylnitrate (0.5 mg/kg). Reserpine was administered simultaneously with atropine and 2 hours later grid shocks were given for 4 hours.

DISCUSSION

Results obtained in the present experiments show that adequate doses of monoamine precursors, i.e. 5-HTP and dopa, administered repeatedly, markedly inhibited ulcer formation by reserpine and grid shock, although serotonin and norepinephrine caused negligible inhibition. Bertler *et al.* (6) reported that dopa could enter into the brain though dopamine could not enter. 5-HTP can also penetrate into the brain and increase the serotonin content in the brain as well as in the peripheral tissues, such as liver, kidney and intestine (7). In reserpinized mice, serotonin content in the brain was increased to the normal level or higher by intraperitoneal administration of 5-HTP (8). Iversen (9) has described that in reserpinized tissue the uptake of catecholamines can occur at a normal rate, but since the accumulated catecholamines can no longer be retained at intraneural storage sites they are rapidly metabolized and leave the tissue. That 5-HTP and dopa had penetrated into the brain and retained the brain monoamine contents at a normal level was evident from the behavioral observation in the present experiments where sedateness caused by 0.1 mg/kg of reserpine disappeared almost completely during or after the first administration of 5 mg/kg of 5-HTP or dopa and sometimes slight excitaiton was observed.

These findings strongly suggest that reserpine and grid shock caused gastrointestinal ulcers by a mechanism which involved the decrease of monoamines, especially in the central nervous system. Kido (1) has reported that ulcers could be produced when grid shock was given after the tissue monoamines had been exhausted by reserpine but they could not be produced when the shock was given during the release of monoamines by reserpine. The author previously observed that serotonin contained in epithelial cells of the gastrointestinal tract of the cat was hardly decreased by 0.1 mg/kg of reserpine alone but could be easily decreased by the same dose of reserpine when followed by grid shock (4).

It has already been postulated by some investigators that monoamines released by reserpine, especially in the central nervous system, play an important role in ulcer formation by reserpine. Haverback and Bogdanski (10) reported that serotonin administered to rats in a dose of 300 mg/kg produced haemorrhagic mucosal erosions in the glandular portion of the stomach and formation of these lesions was prevented by pretreatment with atropine sulfate. Blackman *et al.* (11) also reported that serotonin as well as reserpine caused gastric haemorrhages in normal mice but not in the vagotomized mice and they concluded that reserpine-like drugs caused gastric haemorrhage by a mechanism which involved the liberation of central monoamines. These findings appear to show that liberated monoamines act to produce gastric lesions. However, they could not interpret the preventive action of MAO inhibitor, iproniazid, on ulcer formation by reserpine. Garrattini and Valzelli (12) mentioned that it still has to be established whether this protection is related to the block of MAO or to the direct antiulcer effect of iproniazid.

In the present study it was found that a potent MAO inhibitor, isocarboxazid, could reduce ulcer formation by reserpine and grid shock to about half, and an increase of the dose resulted in disappearance of the protective action. This protective action of MAO inhibitors on ulceration by reserpine, with or without grid shock, may well be explained by the mechanism mentioned above that the decrease of monoamines by reserpine and grid shock caused ulceration.

On the other hand, serotonin and norepinephrine failed to prevent ulcer formation. Since these compounds could increase their contents in the peripheral tissues but not in the brain, it seems that retention of the central monoamine levels at normal levels is required for the protection of ulcer formation by reserpine and grid shock. It is also supported by the fact that tetrabenazine combined with grid shock produced severe ulcers (5). However, that peripheral monoamines participate in this kind of ulcer formation is not neglected for, as already reported (5), syrosingopine could produce ulcers at a dose which failed to induce sedation when combined with grid shock. Thus, it is concluded that local intrinsic factors and disturbance of central integrative function caused gastrointestinal lesions through trophic and vascular disturbance.

Ulceration by reserpine and grid shock was prevented by atropine methylnitrate, similar as that contributed by vagotomy. Some investigators (11, 13) have shown that ulcers produced by large doses of reserpine or serotonin were prevented by atropine and other parasympatholytics. These data suggest the importance of the parasympathetic system in this kind of ulcer formation. In regard to this problem, Brodie *et al.* described that reserpine activated the central parasympathetic discharges.

On the other hand dibenamine slightly decreased ulcer formation. This may be explained by its antisecretory effects (14).

The ulceration described in the present study involved two factors, i.e. reserpine and grid shock. Among the factors concerning this kind of ulceration those dealing with the effects of reserpine may be interpreted in terms of a decrease of monoamines, especially in the central nervous system. The role of grid shock is still to be established. The fact

that grid shock induces emotional responses similar to those induced by hypothalamic stimulation suggests that the hypothalamus is involved in one of the important factors in this kind of ulceration. This is also suggested by the observation of Kido that hypothalamic stimulation in reserpine pretreated cats caused ulceration similar to that produced by reserpine and grid shock. There may be significance to the emotional responses common to hypothalamic stimulation and grid shock. However, in the preliminary experiments it was observed that sonic stimulation given by the same schedule as grid shock used in these experiments to reserpinized cats caused similar ulcerations, though emotional excitement was not observed. Further investigation on the role of grid shock in this kind of ulceration is needed.

SUMMARY

Effects of monoamines, their precursors, MAO inhibitor and blocking agents of the autonomic nervous system on ulcer formation by reserpine and grid shock were investigated. 5-HTP and dopa in a total dose of 50 mg/kg markedly inhibited ulcer formation by reserpine in 0.1 mg/kg with grid shock, but failed to prevent in a total dose of 10 mg/kg. Neither serotonin nor norepinephrine could prevent the same ulceration as markedly as 5-HTP or dopa. Atropine methylnitrate also markedly inhibited ulcer formation in doses more than 0.5 mg/kg, but the inhibitory effect of dibenamine hydrochloride at a dose which could completely inhibit the pressor response to norepinephrine was slight. From these findings it is concluded that reserpine and grid shock caused gastrointestinal ulcers by a mechanism which involved a decrease in monoamine content in the central nervous system. The participation of monoamines in the peripheral tissues should not be excluded, but it may be merely partial.

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