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MECHANISM OF ACTION OF RESERPINE IN PRODUCING GASTRIC HAEMORRHAGE AND EROSION IN THE MOUSE

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Gastric haemorrhage was produced regularly in mice within 6 hours of the subcutaneous injection of a large dose (2 to 10 mg./kg.) of reserpine or of deserpidine. Rescinnamine, syrosingopine (SU-3118), and tetrabenazine (Ro 1-9569) were less active. Gastric haemorrhage was also produced within 6 hours when 5-hydroxytryptamine (10 mg./kg.) was injected every half-hour. Neither reserpine nor 5-hydroxytryptamine produced gastric haemorrhage in mice which had been vagotomized by tying the oesophagus at the cardio-oesophageal junction or which had been pre-treated with iproniazid. Amphetamine was less effective than iproniazid in preventing gastric haemorrhage after reserpine, and the following drugs were ineffective: cocaine, methyl phenidate (Ritalin), amarin, caffeine, nikethamide, lysergic acid diethylamide and its 2-bromo derivative (BOL148). Gastric haemorrhage was not observed in mice which had been given substantial doses of atropine or of hexamethonium before reserpine. The incidence of haemorrhage was substantially reduced by treatment with an antacid mixture. It is concluded that reserpine-like drugs cause gastric haemorrhage by a mechanism which has an important central component and which involves the liberation of 5-hydroxytryptamine.

Haverback and Bogdanski (1957) have shown that gastric erosion was produced in rats within 18 hr. of the injection of large doses of 5-hydroxytryptophan. Reserpine in large doses had a similar effect (Benditt and Wong, 1957; Haverback and Bogdanski, 1957; Blackman, Campion, and Fastier, 1958). Some of the pharmacological actions of both these drugs appear to be mediated by 5-hydroxytryptamine. Reserpine liberates 5-hydroxytryptamine from platelets and certain other parts of the body, and interferes with the uptake of 5-hydroxytryptamine, while 5-hydroxytryptophan is the natural precursor to 5-hydroxytryptamine (Pletscher, Shore, and Brodie, 1955; Page, 1958). Therefore, when we observed in experiments on mice that large doses of reserpine produced gastric haemorrhage and melaena within a few hours (Blackman *et al.*, 1958), we supposed at first that this was a peripheral effect brought about by an increase in "free" 5-hydroxytryptamine. The possibility that it was a central effect was raised by our observation that reserpine did not produce gastric haemorrhage in mice which had been vagotomized at the cardio-oesophageal junction. Another observation at variance with our original hypothesis was that reserpine did not produce gastric haemorrhage in mice which had been previously treated with iproniazid (Blackman *et al.*, 1958). Since iproniazid is a powerful inhibitor of mono-amine oxidase and is believed to delay the destruction of 5-hydroxytryptamine in the body (Page, 1958), it would be expected to enhance rather than antagonize the harmful effect of reserpine on the gastric mucosa, were the erosion brought about by the local action of 5-hydroxytryptamine. These anomalous findings induced us to carry out the experiments reported here.

METHODS

Male mice weighing 30 to 40 g. were used. During the 24 hr. period preceding an experiment, they were fed only on milk and water. They were killed at the end of an experiment by breaking their necks. The abdomen was then opened and the stomach examined for the presence of blood and the intestine for melaena. Doubtful results were recorded as negative.

Vagotomy was performed under ether anaesthesia. When the vagi were cut in the neck, adverse respiratory effects were usually obtained not long afterwards. It was therefore thought preferable to

vagotomize by ligating the oesophagus as close to the diaphragm as was possible. This procedure was always used in these experiments because we found that separating the vagi from the oesophagus was impracticable. Control groups of mice were subjected to a mock operation in which the abdomen was opened and the intestines were handled to the same extent as in the vagotomized animals.

For the estimation of gastric hydrochloric acid, mice were anaesthetized with ether. The abdomen was opened and the oesophagus tied just above the cardiac sphincter (if this had not already been done in the course of vagotomy). Next, a cannula was inserted into the first part of the duodenum, pushed through the pyloric sphincter into the stomach, and tied in position. The stomach contents were washed out with about 10 ml. of distilled water by means of a syringe fitted with a fine-gauge needle which was pushed through the stomach wall. The stomach washings were filtered. After the filtrate had been made up to a volume of 100 ml., 25 ml. portions of this were titrated against 0.01 N-NaOH using phenolphthalein as an indicator.

The antacid used was usually an aluminium hydroxide gel (Amphogel, Wyeth) diluted with distilled water to the extent specified in the text. The antacid solution was injected into the lower oesophagus by means of a fine lubricated polythene tube. The mice of control groups were given distilled water in equal volume by this route.

All the drugs used except amarin were obtained from commercial sources. Reserpine and related compounds were brought into solution with the aid of ascorbic acid (up to 1%).

RESULTS

Gastric Haemorrhage after Reserpine

Reserpine given subcutaneously in a dose of 10 mg./kg. almost invariably caused gross gastric haemorrhage within 6 hr. In 8 successive experiments, only 2 out of 45 mice failed to show gastric haemorrhage. Multiple erosions with bleeding vessels were observed often. Melaena was seen in most mice. Haemorrhage was observed in some mice killed within 30 min. of treatment with reserpine. The incidence of gastric haemorrhage remained high when the dose of reserpine was reduced to 2.5 mg./kg. (Table I). With both doses of reserpine the mice were sedated within 30 min.; they had marked ptosis and some piloerection; and they appeared to suffer abdominal discomfort, some mice having diarrhoea.

TABLE I

INCIDENCE OF GASTRIC HAEMORRHAGE IN GROUPS OF 5 MICE 18 HR. AFTER THE SUBCUTANEOUS INJECTION OF RESERPINE AND RELATED COMPOUNDS

Sedation: ++, marked; + slight; − none.

Drug	2·5 mg./kg.		10·0 mg./kg.	
	Degree of Sedation	Incidence of Haemorrhage	Degree of Sedation	Incidence of Haemorrhage
Reserpine ..	++	5/5	++	5/5
Deserpidine ..	++	5/5	++	5/5
Rescinnamine ..	−	0/5	++	4/5
Syrosingopine ..	−	0/5	+	3/5
Tetrabenazine ..	−	1/5	+	1/5

Effect of Vagotomy by Tying the Oesophagus.—Mice which had been vagotomized at the cardio-oesophageal junction and another group upon which a mock vagotomy had been performed were given reserpine (10 mg./kg.) subcutaneously. Both groups of mice were sedated and had ptosis and piloerection within 30 min. of the injection of reserpine. Most mice showed signs of abdominal discomfort. Nevertheless, whereas gastric haemorrhage was obtained in 5 out of 6 control mice, it was not obtained in any of the 6 vagotomized mice (Table II).

TABLE II

EFFECTS OF VARIOUS TREATMENTS ON INCIDENCE OF GASTRIC HAEMORRHAGE 6 HR. AFTER THE ADMINISTRATION OF RESERPINE OR OF 5-HYDROXYTRYPTAMINE TO MICE

The first of each set of results gives the number of mice out of a group of 6 showing gastric haemorrhage. Reserpine was given subcutaneously in a single dose; other drugs were given in divided doses. Atropine sulphate (total 12 mg./kg.), hexamethonium bromide (total 120 mg./kg.), and 5-hydroxytryptamine (5-HT) (total 120 mg./kg.) were each given in a series of 12 doses at 30 min. intervals. Iproniazid was given in 2 doses each of 100 mg./kg., the first 24 hr. before and the second 30 min. before the injection of reserpine.

Treatment		Reserpine		5-HT	
	Dose (mg./kg.)	Treated	Control	Treated	Control
Vagotomy ..	—	0/6	5/6	1/6	5/6
Atropine	12 × 1	1/6	6/6	—	—
Hexamethonium	12 × 10	0/6	6/6	—	—
Iproniazid ..	2 × 100	0/6	6/6	1/6	5/6

Effect of Atropine and Hexamethonium.—These drugs were given to produce a "chemical" vagotomy. Each was injected subcutaneously 30 min. before the reserpine was given and then at 30 min. intervals during the 6 hr. that the mice were under the influence of reserpine. As may be seen from Table II, both drugs were highly effective in preventing gastric haemorrhage after reserpine.

Influence of Gastric Acidity.—The possibility that vagotomy could prevent the occurrence of gastric haemorrhage after reserpine by diminishing the secretion of acid was next considered. It was found that the free acid content of the stomachs of mice which had been vagotomized before treatment with reserpine was actually greater than that of normal mice treated

with reserpine. A second experiment gave substantially the same results as those summarized in Table III.

TABLE III
GASTRIC ACID CONTENT AND INCIDENCE OF GASTRIC HAEMORRHAGE IN MICE 2 HR. AFTER RESERPINE

Reserpine (10 mg./kg.) was injected subcutaneously. Those mice which had been vagotomized were allowed to recover fully from ether anaesthesia before they were given reserpine.

Treatment	Mean HCl 10^{-5}M (with s.e.)	Incidence of Haemorrhage
—	1·5 (±0·2)	0/5
Reserpine	1·8 (±0·4)	3/5
,, +vagotomy ..	3·5 (±0·9)	0/5

To see whether the incidence of gastric haemorrhage after reserpine could be reduced by diminishing gastric acidity, experiments were performed in which antacids were introduced into the stomach. Only 2 out of 6 mice which had received at 45 min. intervals eight 0.1 ml. doses of amphogel diluted 1:5 with water showed gastric haemorrhage when killed 6 hr. after the injection of reserpine (5 mg./kg.), whereas all the mice of the control group did. In another experiment in which a stronger solution of the antacid was used (amphogel diluted 1:2 with distilled water), none of the treated mice showed gastric haemorrhage. In this experiment, however, the reserpine was less effective than usual, only 2 of the 5 controls showing haemorrhage. Four 0.1 ml. doses of a dilute (1:10) amphogel solution gave no protection, the incidence of haemorrhage being 6 out of 6 for both the mice which had been given amphogel and for those which had been given saline. For another group of mice which had been treated at the same time with sodium bicarbonate (given in hourly doses of 0.1 ml. of a 0.5% solution) the incidence of haemorrhage was 4 out of 6.

Gastric Haemorrhage after other Drugs

Reserpine-like Drugs.—Two of the other compounds tested, deserpidine and rescinnamine, are also *Rauwolfia* alkaloids. Syrosingopine (SU - 3118; methyl *O* - 4 - ethoxycarbonyl - 3:5 - dimethoxybenzoylreserpate) is a semi-synthetic compound closely related to reserpine. Tetrabenazine (Ro 1-9569; 3-*iso*butyl-1:2:3:4:6:7-hexahydro - 9:10 - dimethoxy - 11bH - benzo[*a*] - quinolizine-2-one) is not at all closely related to reserpine chemically but resembles it pharmacologically (Pletscher, 1957).

When given in the same quantities as reserpine, deserpidine showed comparable activity (Table I). The other three compounds were less active, not only in producing gastric haemorrhage, but also in producing sedation.

5-Hydroxytryptamine.—When given to mice in large divided doses (10 mg./kg. subcutaneously every 30 min.), 5-hydroxytryptamine caused severe gastric haemorrhage in 4 out of 6 mice examined 3 hr. later and in 5 out of 6 mice examined 6 hr. later. The mice were not obviously sedated at any stage of the experiment. Other experiments with 5-hydroxytryptamine were performed on mice which had been subjected to either a real or a mock vagotomy. After 6 hr. treatment with 5-hydroxytryptamine (given in 12 doses each of 10 mg./kg. at 30 min. intervals), gastric haemorrhage was observed in only 1 out of 6 vagotomized mice but in 5 out of 6 control mice (Table II).

Pilocarpine.—The effect of vagotomy in the experiments just described suggested that increased vagal tone might play an important part in the production of gastric haemorrhage. The effect of a parasympathomimetic drug, pilocarpine, was therefore studied in both normal and vagotomized mice. Pilocarpine was given subcutaneously in 5 divided doses, each of 10 mg./kg., over a period of 4 hr. The amount given was comparable with that used by Underhill and Freiheit (1928) for the production of gastric erosion in rabbits. Of the 6 normal mice so treated, 5 showed gastric haemorrhage. All 6 vagotomized mice showed gastric haemorrhage and melaena.

Effect of Iproniazid

Previously (Blackman *et al.*, 1958) we performed some experiments with iproniazid at a time when we had little 5-hydroxytryptamine, and thought that we could enhance the effect of a moderate dose by treatment with an amine oxidase inhibitor. We found that gastric haemorrhage was not produced in mice which had been given large doses of iproniazid before treatment with 5-hydroxytryptamine was begun; nor was haemorrhage produced in mice which had been given iproniazid before reserpine (Table II). This made us wonder whether the gastric erosion produced by reserpine could be prevented by treatment with drugs of such types as central stimulants and amine oxidase inhibitors. Experiments were therefore performed with the substances listed in Table IV.

In the doses which we employed, only one of these substances reduced the incidence of gastric haemorrhage after reserpine, namely amphetamine. The central depressant action of

TABLE IV

EFFECTS OF VARIOUS TREATMENTS ON THE INCIDENCE OF GASTRIC HAEMORRHAGE 6 HR. AFTER THE ADMINISTRATION OF RESERPINE

All mice were given reserpine (2 mg./kg.) subcutaneously. Marked sedation is indicated as ++, mild sedation (lasting about an hour) by + and − indicates no sedation. The other drugs mentioned were given by subcutaneous injection usually in divided doses. For example, caffeine was given in 3 doses, each of 15 mg./kg., at 2 hr. intervals. LSD25=lysergic acid diethylamide and BOL148 is the 2-bromo derivative of this compound.

Treatment	Dose (mg./kg.)	Degree of Sedation	Incidence of Haemorrhage	
			Treated	Untreated
Iproniazid	2 × 100	−	0/6	6/6
Amphetamine ..	3 × 2·5	−	1/6	5/6
LSD25	5 × 3	−	6/6	6/6
Cocaine	2 × 7·5	+	6/6	3/6
Caffeine	3 × 15	+	6/6	5/6
Methyl phenidate ..	4 × 100	+	6/6	6/6
Nikethamide ..	5 × 100	++	5/6	6/6
Amarin	1 × 10	++	6/6	6/6
BOL148	5 × 10	++	6/6	6/6

reserpine was antagonized fairly effectively by amphetamine in the dose used (which was sufficiently large to kill some of the mice). Lysergic acid diethylamide antagonized the sedative action of reserpine even more effectively than did amphetamine; mice previously treated with lysergic acid diethylamide appeared to be hyperactive. However, these mice showed worse gastric haemorrhage than did mice given reserpine alone. Lysergic acid diethylamide was given in large divided doses because it is known to be rapidly metabolized in mice. 2-Bromolysergic acid diethylamide had no obvious effect on the response to reserpine. The central depressant action of reserpine was not antagonized strongly by caffeine, cocaine, or methyl phenidate (Ritalin) and was hardly affected by amarin or nikethamide.

DISCUSSION

Considerable evidence has been obtained in support of the hypothesis, proposed first by Brodie and his co-workers, that the pharmacological activity of reserpine is due in large measure to its ability to increase the amount of "free" 5-hydroxytryptamine present in the body (Page, 1958). The possibility must therefore be considered that reserpine in large doses produces gastric haemorrhage and erosion in the mouse and the rat by releasing 5-hydroxytryptamine from physiologically inert combinations in amounts sufficient to have a local deleterious effect.

That this action of reserpine is mediated by 5-hydroxytryptamine is strongly suggested by our finding that 5-hydroxytryptamine itself, if it is given repeatedly in large doses, produces gastric haemorrhage in the mouse. Other workers (Wilhelmi, 1957; Hedinger and Veraguth, 1957) have shown that 5-hydroxytryptamine in large doses causes gastric ulceration in the rat. The 5-hydroxytryptamine precursor, 5-hydroxytryptophan, has also been shown to produce gastric mucosal erosion in the rat (Haverback and Bogdanski, 1957).

Haverback and Bogdanski (1957) observed that lesions after the administration of 5-hydroxytryptophan were fewer or even absent if the rats had been atropinized. We have found similarly that the incidence of gastric haemorrhage after reserpine is greatly reduced by previous treatment with atropine or hexamethonium. Neither reserpine nor 5-hydroxytryptamine produced gastric haemorrhage in the great majority of vagotomized mice (Table II).

One interpretation of our results with vagotomized mice is that 5-hydroxytryptamine may produce by a peripheral action a mucosal ischaemia which is exacerbated by normal vagal activity. Wilhelmi (1957) found that the ulcerogenic action of 5-hydroxytryptamine in rats could be antagonized by lysergic acid diethylamide, which is known to be a powerful antagonist of the vasoconstrictor action of 5-hydroxytryptamine. Lysergic acid diethylamide has other important actions. Thus mice given this drug after reserpine became hyperactive. We were unable to reduce the incidence of gastric haemorrhage after reserpine in mice by treatment with lysergic acid diethylamide or with its 2-bromo derivative.

The observation that gastric haemorrhage was hardly ever produced by reserpine in vagotomized mice could have been readily explained had it been found that the amount of hydrochloric acid in the stomachs of reserpine-treated mice was decreased by vagotomy. However, no decrease was observed (Table III). The role of gastric acidity in the production of mucosal lesions was made clearer by our experiments with antacids; we found that the repeated oral administration of antacids could reduce substantially the incidence of haemorrhage in reserpine-treated mice. It would thus appear that, whatever be the primary cause of the mucosal lesion, the acid in the stomach helps the development of gross haemorrhage.

That reserpine produces gastric haemorrhage by virtue of a central action is suggested, not only by the results obtained with vagotomized mice, but also by those obtained with reserpine-like drugs;

these had to be given in doses sufficient to produce characteristic central effects for haemorrhage to be obtained regularly with them (Table I).

Haemorrhages, erosions, and acute ulcers of the stomach and duodenum have been obtained by a variety of procedures which cause intracranial damage (Ivy, Grossman, and Bachrach, 1950; Spira, 1955). While it remains to be shown that these acute lesions of the digestive tract result from a specific derangement of nervous function, at least it is clear that they can be produced by mechanisms which affect the central nervous system primarily. Hence it is not unreasonable to suppose that the liability of reserpine-like compounds to cause gastric haemorrhage when they are given in large amounts may be related to their ability to depress the central nervous system in such a way that discharge through the sympathetic division is depressed relative to that through the parasympathetic division. Although no one has shown satisfactorily that *chronic* peptic ulcers can be produced by prolonged stimulation of the vagi or by the prolonged administration of parasympathomimetic drugs, numerous workers have shown that mucosal haemorrhage can be readily produced by simulated vagotonia. Our results with pilocarpine bear out those of several other investigators whose work is discussed by Ivy *et al.* (1950). Why gastric haemorrhage should be obtained under these conditions remains obscure. Ivy *et al.* (1950) think it significant that the acute lesions which are produced in the stomach by interference with the nervous mechanism almost always begin as a focal haemorrhage or ischaemia. It seems that "the vascular, rather than the acid factor, initiates the mucosal injury . . . the primary lesion appears to be the same whatever part of the nervous system is damaged and regardless of whether the disturbance consists of irritation or interruption."

The ability of iproniazid to prevent the production of haemorrhage by either reserpine or 5-hydroxytryptamine provides further support for the view that the ulcerogenic action of reserpine is mediated by 5-hydroxytryptamine. Why iproniazid should act thus is difficult to explain. We originally expected iproniazid to intensify rather than antagonize the ulcerogenic action of 5-hydroxytryptamine, by increasing the amount of free 5-hydroxytryptamine present in the gut through inhibition of mono-amine oxidase. It has since become clear that iproniazid can influence the metabolism of 5-hydroxytryptamine by other means than inhibition of mono-amine oxidase. Thus it can inhibit the decarboxylation of 5-hydroxytryptophan, possibly by virtue of an ability to compete with the co-enzyme pyridoxal-5-phosphate (Westermann, Balzer, and Knell, 1958; Palm, 1958). Moreover, it has been shown that iproniazid may have little effect on peripheral depots of 5-hydroxytryptamine even when given in amounts which produce a two- to three-fold increase in endogenous brain 5-hydroxytryptamine (Udenfriend, Weissbach, and Bogdanski, 1957). We think it significant that previous treatment with iproniazid so changes the response of experimental animals to reserpine that the latter drug causes excitement and hyperactivity instead of sedation (Shore and Brodie, 1957), an observation which was confirmed by us. The fact that some central actions of reserpine can be antagonized by iproniazid makes it less surprising that the ulcerogenic action can also be antagonized by iproniazid, at any rate so long as the latter action is believed to have an important central component.

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