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Histamine formation in rat gastric mucosa and lung after injecting reserpine or adrenaline

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1. In rats the effect of reserpine, adrenaline and gastrin on the histamine forming capacity (HFC) of gastric mucosa and lung was examined.

2. Intraperitoneal reserpine as well as subcutaneous adrenaline produced a great increase in HFC of gastric mucosa but a great decrease in HFC of lung. After reserpine the HFC of lung was almost abolished.

3. As the HFC changes produced by reserpine occurred also after demedullation of the suprarenals—the changes in the mucosa were in fact accentuated—release of the medullary hormones by the reserpine does not account for these changes.

4. Subcutaneous gastrin caused a great increase in HFC of the gastric mucosa but did not affect the HFC in lung.

5. The possible role of release of gastrin and corticosteroids for the reserpine-induced HFC changes is discussed because it is known that reserpine releases gastrin and corticosteroids and that cortisone produces HFC changes in gastric mucosa and lung.

In rats, reserpine not only causes acid gastric secretion which is strongly inhibited by vagotomy and by the ganglion blocking agent chlorisondamine (Shore, 1965), but also reduces the histamine content of the stomach wall (Haverback & Wirtschafter, 1962 ; Kim & Shore, 1963). This suggested that reserpine might affect histamine formation because in other conditions in which the histamine content of the stomach wall is reduced its histamine forming capacity (HFC) is increased as well. Kahlson, Rosengren, Svahn & Thunberg (1964), for instance, showed that in rats, feeding and gastrin injections reduced the histamine content of the gastric mucosa and increased its HFC. They therefore postulated a feed-back mechanism between histamine release and HFC. In anaphylaxis, too, an increased HFC was found in those tissues from which histamine was released (Kahlson, Rosengren & Thunberg, 1966).

The present experiments show that, in rats, intraperitoneal injections of reserpine which reduce the histamine content of the gastric mucosa do in fact increase its HFC. The effect of reserpine on the HFC of lung was studied at the same time because it is known that its HFC may undergo the opposite change from that of the gastric mucosa. Schayer (1956) found that after cortisone, histamine formation was increased in the stomach but decreased in the lung.

As reserpine is known to release the catecholamines stored in the tissues (Shore, 1962) its ability to influence the HFC of tissues might be mediated by this action. To test this possibility, rats were either given subcutaneous injections of adrenaline to find out if adrenaline would reproduce the reserpine induced changes in HFC, or their suprarenals were demedullated to find out if this would prevent these changes. But reserpine also releases gastrin. This was shown in cats by Emås & Fyrö (1965). The release was brought about by central vagal excitation and resulted in depletion of the gastrin stores. Further, Rosengren & Svensson (1969) found that gastrin as well as vagus stimulation increased the histidine decarboxylating activity of the gastric mucosa. The reserpine induced changes in HFC might therefore also be mediated by gastrin, a possibility tested in the present experiments by examining the effect of subcutaneous gastrin injections in rats on the HFC of gastric mucosa and lung.

Methods

Two strains of 3–6 month old female rats weighing between 160 and 200 g were used; the albino Sprague-Dawley strain to determine the time course of HFC changes (Table 1 and Fig. 1) and a black-white strain inbred at the Institute of Physiology, Lund, for all other experiments.

The rats were fasted for 16 hr before removal of the tissues. The animals were killed by a blow on the head and bled, lung and stomach were removed and the stomach was cleaned by washing with 0·9% NaCl solution, blotted with gauze and the mucosa of the parietal cell containing region was collected by scraping with a scalpel.

Determination of histamine forming capacity (HFC)

The original method of Schayer was adapted for use in our laboratory (Kahlson, Rosengren & Thunberg, 1963). It involves the following steps. The minced tissues were incubated for 3 hr at 37° C under nitrogen in beakers containing 150–200 mg of tissue, 40 μg of 2-ring-(^{14}C)-labelled-L-histidine (base), 10^{-4}M aminoguanidine sulphate, 10^{-1}M sodium phosphate buffer, pH 7·4, and 0·2% glucose, all made up to a final volume of 3·2 ml. At the end of incubation, carrier histamine and perchloric acid were added. After filtration radioactive histidine was separated from radioactive histamine on an ion exchange resin and after conversion of the histamine to pipsyl histamine the radioactivity of formed histamine was determined at infinite thickness in a flow counter. With the (^{14}C)-histidine and measuring equipment used 1 μg (^{14}C)-histamine formed corresponded to about 5,000 c.p.m. Values are expressed as ng of (^{14}C)-histamine formed per g of tissue.

Removal of the adrenal medulla

In an aseptic operation under ether anaesthesia, the suprarenals were approached from the back, a small incision was made in each of the suprarenals, and the medullary tissue was expelled. In the week after operation the rats were given 1% NaCl to drink, and the actual tests were carried out 6 weeks later. Sham operations using similar surgical procedures without demedullating the suprarenals were performed in control rats.

Drugs

Reserpine in the form of Serpasil (Ciba), 2·5 mg/ml., and L-adrenaline (Fluka AG) were diluted with 0·9% NaCl-solution to the desired concentration and injected shortly after dilution. Gastrin II was obtained by the courtesy of Professor Gregory, Liverpool. All injections were made in a volume of 0·1–0·2 ml. and control rats received injections of the same volume of 0·9% NaCl solution.

Results

Reserpine

In preliminary experiments four intraperitoneal injections of 2 mg/kg reserpine were given at 12 hr intervals. When the rats were killed 12 hr after the last injection, the HFC of the gastric mucosa was increased 2 to 3 times, whereas the HFC of the

TABLE 1. *HFC of gastric mucosa and lung in rats at different times after an intraperitoneal injection of reserpine (2 mg/kg)*

Time after injection	HFC ng/g histamine formed	
	Gastric mucosa	Lung
Control	3,500±1,200 (13)	190±99 (13)
1 hr	3,200±1,500 (6)	260±210 (6)
3 hr	2,200±1,100 (6)*	170±150 (6)
5 hr	4,200±2,000 (6)	75±63 (6)†
12 hr	8,200±1,800 (6)‡	20±7·0 (6)‡
24 hr	8,800±2,600 (6)‡	20±3·6 (5)‡
48 hr	3,100±1,000 (6)	210±100 (6)

* $P<0.05$; † $P<0.02$; ‡ $P<0.01$.
The figures give the means and standard deviations and those in parentheses refer to the number of animals.

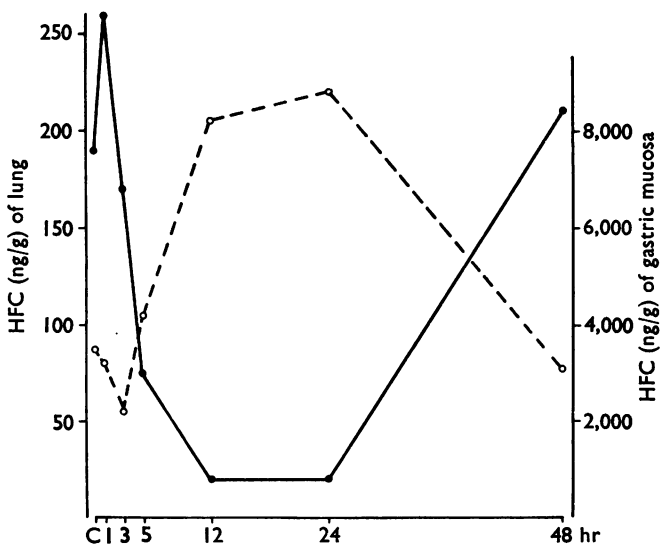

FIG. 1. Changes in gastric mucosa (○- - -○) and lung (●——●) HFC resulting from a single intraperitoneal injection of reserpine, 2 mg/kg.

small intestine (all three layers) was slightly reduced. Since, at the time of killing, the rats had severe diarrhoea and were found *post mortem* to have haemorrhages in the stomach, only single intraperitoneal injections of 2 mg/kg were given in subsequent experiments and the rats were killed at different times after the injections. The changes produced in the HFC of the gastric mucosa and of the lung are shown in Table 1.

Three hours after the injection, the HFC of the gastric mucosa had fallen by about 40%, it then rose and after 24 hr had reached a value of about 250% of the control value; after another 24 hr the HFC had returned to normal. The HFC of the lung, on the other hand, had fallen by about 60% after 5, and by about 90% after 12 hr; it remained at this low value for the next 12 hr and then returned to normal after another 24 hr. The strikingly different pattern of changes produced in the HFC of gastric mucosa and lung is shown in the graphs of Fig. 1.

Adrenaline

A subcutaneous injection of 100 μg of adrenaline per animal affected the HFC of gastric mucosa and lung in the same way as the reserpine. The changes were of the same magnitude but the time course was different. As shown in Table 2, the HFC of gastric mucosa had fallen by about 40% after half an hour. It then rose and reached a value of about 260% of the control value 5 hr after the injection;

TABLE 2. *HFC of gastric mucosa and lung of rats at different times after subcutaneous injection of adrenaline, 100 μg/rat*

Time after injection	HFC ng/g histamine formed	
	Gastric mucosa	Lung
Control	3,800±1,700 (12)	190±81 (12)
15 min	4,000±1,900 (6)	350±180 (6)†
30 min	2,200±730 (6)*	220±76 (6)
1 hr	3,200±2,500 (8)	200±99 (8)
3 hr	3,700±2,600 (6)	110±46 (6)*
5 hr	10,000±3,900 (6)‡	46±15 (6)‡
12 hr	7,500±2,200 (6)‡	97±59 (6)*
24 hr	4,700±3,200 (6)	240±75 (6)

* $P<0.05$; † $P<0.02$; ‡ $P<0.01$.
The figures give the means and standard deviations and those in parentheses refer to the numbers of animals.

TABLE 3. *HFC of gastric mucosa and lung 18 hr after an intraperitoneal injection of reserpine (2 mg/kg) in five sham operated and six demedullated rats*

	HFC ng/g histamine formed	
	Sham operation	Demedullation
Lung	17, 30, 48, 65, 120	12, 13, 15, 15, 18, 23
Mean±S.D.	56±40	16±4
	$P<0.05$	
Gastric mucosa	4,400, 6,500, 6,600, 8,000, 10,000	14,000, 16,000, 18,000, 20,000 31,000
Mean±S.D.	7,100±2,100	20,000±5,900
	$P<0.01$	

then it fell again and was almost normal after 24 hr. The HFC in the lung initially rose, then gradually fell and reached the lowest value 5 hr after the injection ; after 24 hr, the HFC had returned to normal.

Reserpine after demedullation of the suprarenals

Although subcutaneous adrenaline produced changes in HFC of gastric mucosa and lung similar to those produced by an intraperitoneal injection of reserpine, the changes produced by reserpine were not prevented, but, on the contrary, were accentuated by demedullation of the suprarenals. This is shown in Table 3. In the sham-operated rats the changes of HFC produced in the lung were not quite as pronounced as those shown in Table 1. On the other hand, in the demedullated animals reserpine produced changes which were particularly marked in the gastric mucosa. The adrenal medulla thus appears to exert a restraining influence on the reserpine-induced changes in HFC of the tissues.

Gastrin

Repeated subcutaneous injections of gastrin produced about a six-fold increase of HFC in the gastric mucosa but did not affect the HFC in the lung. This is shown in Table 4. The rats had received seven injections of 1 μg of gastrin per animal given at 30 min intervals and were killed 30 min after the last injection.

Discussion

Previous observations from this laboratory have shown that a lowering of the histamine content in the gastric mucosa of rats produced by feeding or gastrin injections is associated with an increase in its HFC (Kahlson *et al.*, 1964). The present finding that reserpine, which reduces the histamine content of gastric mucosa, also increased its HFC provides another instance of this correlation between histamine content and HFC in gastric mucosa and raises the question whether the underlying mechanism by which these changes are brought about is the same in all three conditions.

The additional finding that the HFC of lung decreased and thus underwent a change in the opposite direction from that of gastric mucosa again is not specific for

TABLE 4. *HFC of gastric mucosa and lung in six rats, each given seven subcutaneous injections of 1 μg gastrin at 30 min intervals and in six control rats given seven injections of 0·9% NaCl solution*

	HFC ng/g histamine formed			
	Gastric mucosa		Lung	
	Control	Gastrin	Control	Gastrin
	5,200	24,000	140	160
	4,500	26,000	100	71
	2,700	45,000	100	220
	4,400	27,000	140	80
	4,300	28,000	77	72
	6,000	18,000	140	110
Mean±S.D.	4,500±1,200	28,000±9,100	116±28	119±60

reserpine, since it has been shown also after cortisone (Schayer, 1956). On the other hand, an elevation of gastric mucosal HFC is not always associated with a decrease of HFC in the lung. In the present experiments gastrin was found to increase gastric mucosal HFC without affecting the HFC in the lung. Different mechanisms may therefore be responsible for the HFC changes produced by reserpine in gastric mucosa and lung.

The changes produced by reserpine in the HFC of gastric mucosa and lung could be reproduced by adrenaline injections. Nevertheless, release of the medullary hormones from the suprarenals is not the mechanism responsible for these changes when they are produced by reserpine because they occurred also after demedullation of the suprarenals. In fact they were greatly accentuated in this condition. The mechanism of this potentiation has not been examined.

The pronounced and long lasting elevation of gastric mucosal HFC after injections of reserpine accounts, at least partly, for the acid gastric secretion and for the ulceration found in the gastric mucosa after such injections. The increase in mucosal HFC may well be the result of increased vagal activity as well as of antral activity—that is, of release of gastrin. According to Emås (1965) reserpine stimulates acid gastric secretion even in the absence of vagal and antral influences. It would therefore be interesting to know whether in this condition reserpine still would increase the HFC in gastric mucosa.

Although release of gastrin may account for the elevation of HFC in the gastric mucosa it cannot be responsible for the reduction of HFC in the lung produced by reserpine since gastrin did not affect the HFC in the lung. The effect produced by reserpine in the lung, which amounted to nearly disappearance of its histidine decarboxylating activity, could on the other hand be explained by an action of corticosteroids, because reserpine releases corticosteroids, as shown in the rat by Saffran & Vogt (1960), and, as mentioned earlier, cortisone lowers the HFC in the lung. Since cortisone increases the HFC in gastric mucosa as well, the HFC elevation produced by reserpine in this tissue could be a combined effect of gastrin and corticosteroids, the reduction of HFC produced in the lung an effect of the corticosteroids alone. Further speculation about the mechanisms which may be responsible for the HFC changes produced by reserpine is not justified at present; nor is it possible to explain satisfactorily why, in gastric mucosa and lung, the major change is preceded by a transient change in the opposite direction.

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