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NICOTINE-INDUCED ALTERATIONS IN HISTAMINE METABOLISM IN THE RAT

BY

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Nicotine is known to be a very potent pharmacological compound and a strong poison (for references see Larson, Haag & Silvette, 1961). Its actions are complex and unpredictable, involving many structures or systems. The wide range of its actions is partly due to an excitatory or inhibitory action on autonomic ganglia (Langley & Dickinson, 1889).

Dale & Laidlaw (1912) found that the pressor response to nicotine in the cat was due partly to liberation of noradrenaline from the adrenal glands. Subsequently the release of catecholamines by nicotine has been thoroughly investigated by Westfall (1965) and Armitage and Milton (1965).

In some investigations on the effects of nicotine, a possible participation of histamine has been considered. In the dog heart-lung preparation Aviado, Samarick & Folle (1966) found a release of histamine from the lung by cigarette smoke. Schievelbein & Werle (1967) showed that histamine could be released from rabbit thrombocytes *in vitro* by nicotine and other tobacco alkaloids. In man, Werle & Effkeman (1940) found that strong smokers had higher values of blood histamine than non-smokers.

To study the effect of nicotine on histamine metabolism we have used the female rat, because in this species changes in the rate of urinary excretion of free histamine parallel changes in the rate of histamine formation, which particularly in the gastric mucosa is of high magnitude (Kahlson, Rosengren, Svahn & Thunberg, 1964).

METHODS

Twenty female white rats of the Sprague-Dawley strain were fed a semisynthetic food essentially free from histamine, 10 g being given daily (Kahlson, Rosengren & Westling, 1958). Eight rats were kept in metabolism cages for collection of urine in 24 hr portions, with a few drops of hydrochloric acid in the collecting vessels. Urinary histamine was determined by bioassay on the guinea-pig ileum as described by Angervall, Bjurö & Westling (1961). Values are given as μg of free histamine base excreted in 24 hr. The identity of the activity investigated was verified by the antihistamine test described by Reuse (1948). Mepyramine, in a final concentration of 0.1 μg/ml., was allowed to act on the strip of gut for 10 sec. This abolished responses to doses of histamine which were 10 times larger than those used in the actual tests. Mepyramine, 0.1 μg/ml., did not diminish the contraction elicited by 4 μg of nicotine base, a dose equiactive with the test dose of histamine (0.02 μg). Nicotine did not sensitize the gut to histamine and its effect was merely additive with that of histamine.

Drugs

The rats kept in metabolism cages were injected subcutaneously once daily with aminoguanidine in a dose of about 20 mg/kg body weight, in order to inhibit histaminase.

Nicotine base in 0.9% NaCl solution (1 mg/ml.) was injected subcutaneously. The animals in metabolism cages received increasing amounts: 0.2 mg once daily for 2 days, 0.4 mg once daily for 2 days, 0.8 mg once daily for 13 days and 0.8 mg three times daily for 16 days. The rats whose tissues were examined *in vitro* received 0.8 mg three times daily for nine days.

The 0.8 mg dose regularly induced convulsions lasting up to 10 min after the injection.

Controls in both groups were injected with 0.9% NaCl solution of the same pH (9.4) and volume as applied to the nicotine solution.

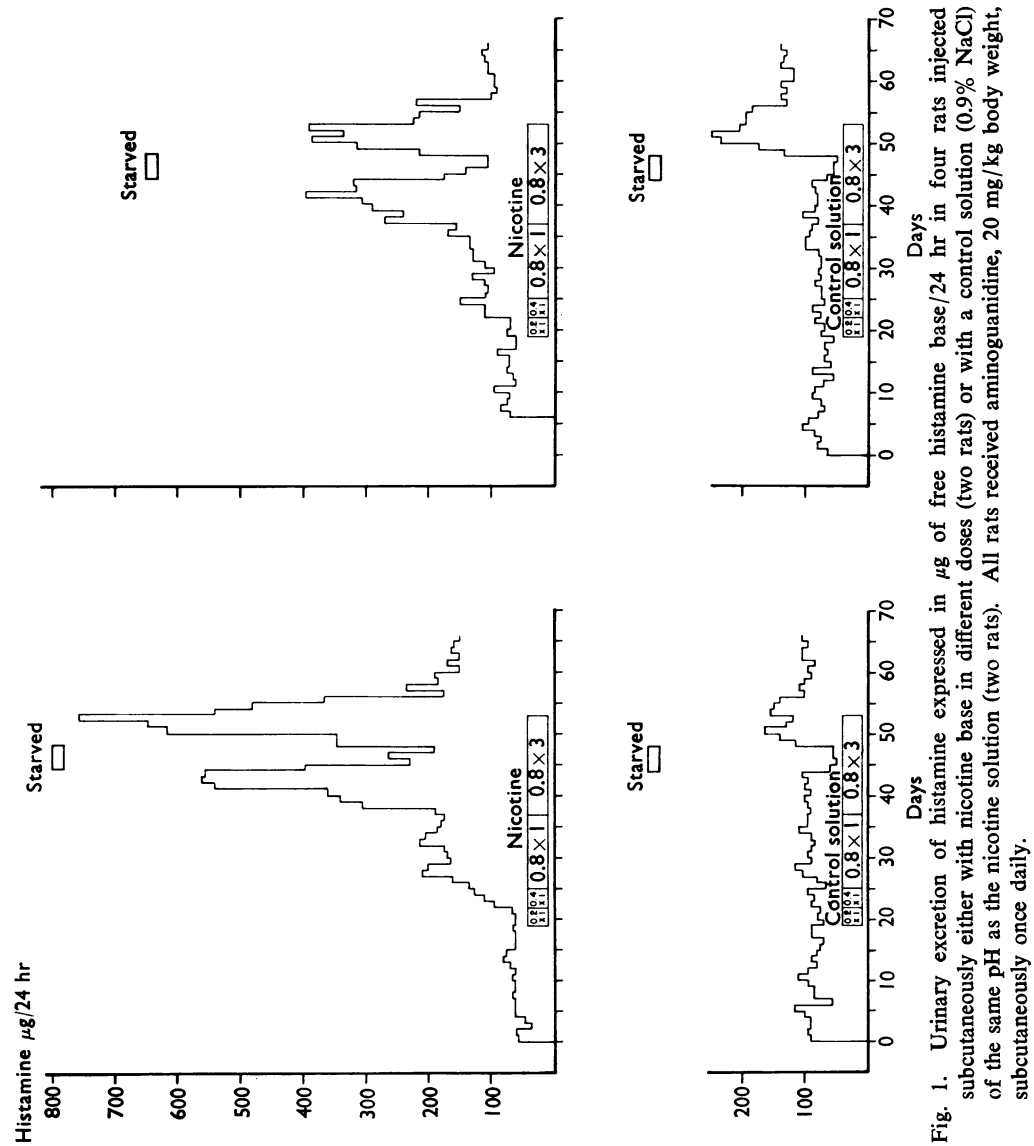

Fig. 1. Urinary excretion of histamine expressed in µg of free histamine base/24 hr in four rats injected subcutaneously either with nicotine base in different doses (two rats) or with a control solution (0.9% NaCl) of the same pH as the nicotine solution (two rats). All rats received aminoguanidine, 20 mg/kg body weight, subcutaneously once daily.

Determination of histamine-forming capacity in vitro

For the *in vitro* studies of histamine-forming capacity, the animals were starved for 24 hr. The animals were killed by a blow on the head 2 hr after the last injection of nicotine or control solution.

The glandular layers of the parietal cell containing part of the stomach were removed by scraping with a scalpel after the opened stomach had been cleaned and pinned flat. Abdominal skin and lungs were also excised. All tissues were minced with scissors.

The rate of histamine formation (HFC, that is, histidine decarboxylase activity) was determined by a procedure fully described by Kahlson, Rosengren & Thunberg (1963). The method involves the following steps. The minced tissues are incubated for 3 hr at 37° C under nitrogen in beakers containing 200–300 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 are added. After filtration radioactive histidine is separated from radioactive histamine on an ion-exchange resin and after conversion of the histamine to pipsylhistamine the radioactivity of formed histamine is determined at infinite thickness in a flow counter. With the [^{14}C]-histidine and the measuring equipment used 1 μg formed [^{14}C]-histamine corresponded to about 5,000 c.p.m.

RESULTS

Nicotine causes an increased histamine excretion in the urine and the effect increases as the dose of nicotine is increased. Similar results were obtained in six rats injected with nicotine, and Fig 1 illustrates the findings in two of them, together with observations on two control rats.

When food is withdrawn for 4 days, urinary histamine excretion drops significantly but is maintained at relatively high values in animals injected with nicotine. In control animals starvation reduces the histamine output below the level during feeding. This is in agreement with earlier findings (Kahlson *et al.*, 1964). Control animals refed after 4 days of starvation display an overshoot phenomenon (Fig. 1). In rats injected with nicotine, refeeding restores the high rate of histamine excretion and the overshoot phenomenon is less impressive.

In a few experiments nicotine tartrate was used. This compound had a weaker effect than the free base on urinary histamine excretion.

TABLE 1

HISTAMINE FORMING CAPACITY (EXPRESSED IN c.p.m.) OF [^{14}C]-HISTAMINE FORMED FROM RING LABELLED [^{14}C]-HISTIDINE IN DIFFERENT TISSUES FROM RATS GIVEN SUBCUTANEOUSLY FOR 9 DAYS 0·8 mg OF NICOTINE BASE THREE TIMES DAILY OR THE SAME VOLUME OF 0·9% NaCl OF THE SAME pH AS THE NICOTINE SOLUTION

In each line figures denote one experiment on the tissue of one animal.

	Stomach		Lung		Abdominal skin	
	Nicotine-treated	Control	Nicotine-treated	Control	Nicotine-treated	Control
Starved	39,085	8,215	292	666	126	159
	53,720	10,410	452	758	130	150
	77,520	10,590				
Starved in cage with wood shavings	73,055	17,065	935	2,976	79	81
	97,870	33,515	240	1,254	123	118
	192,600	77,580	156	1,220	111	158

Table 1 summarizes results obtained on the formation of [^{14}C]-histamine from ring-labelled [^{14}C]-histidine, and shows that it is greatly increased in the stomach of the nicotine-injected rats. Unfortunately six starving rats (three nicotine-injected and three control rats) were kept in cages with wood shavings. They consumed this material and their stomachs were not empty. This would explain the higher mucosal HFC values in these rats, for distension of the stomach results in an elevated mucosal HFC (Kahlson *et al.*, 1964). Nevertheless nicotine was found to elevate HFC markedly in these animals also.

In the lung of rats treated with nicotine the histamine-forming capacity was lower than in controls and in the skin nicotine induced no measurable changes in HFC.

DISCUSSION

Nicotine is excreted into the urine as shown by Werle & Uschold (1948). This does not seem to disturb the bioassay, because with daily subcutaneous injections of nicotine the excretion of the alkaloid is diminishing (Werle & Uschold, 1948). Reuse (1948) and Clark, Rand & Vanov (1965) reported that nicotine contracted the guinea-pig ileum. Moreover, comparing the gut contracting activities of nicotine (Reuse, 1948 ; Clark *et al.*, 1965) and histamine, the amine was found to be 200 times more active than nicotine. Consequently no enhancement of histamine induced contractions of the guinea-pig ileum by nicotine was observed. Assuming that the metabolites of nicotine are not more potent in contracting the gut than is nicotine, it would appear that the amounts of nicotine injected (maximally 2.4 mg/24 hr) could not affect the assay of urinary histamine (about 100–800 μg/24 hr).

In the present study, mepyramine, 0.1 μg/ml., completely abolished the histamine contraction, whereas that of nicotine was seemingly unaffected. Reuse (1948) showed that mepyramine in a concentration of 4 μg/ml. only partly inhibited the response to nicotine tartrate (1 μg/ml.) while mepyramine at a concentration of 0.001 μg/ml. was sufficient to produce a marked and prolonged inhibition of equivalent responses to histamine. The contracting activity of urinary samples was completely suppressed by mepyramine, 0.1 μg/ml., so it appears that histamine was the agent determined.

Great individual variations in the urinary histamine output are likely to be paralleled by corresponding variations in the histamine forming capacity of the stomach, because in the rat the stomach is a major site of histamine formation. Our results show that there is a close relation between the amount of histamine excreted in the urine and the rate of histamine formation in the stomach.

The histamine forming capacity of the stomach is elevated by feeding, distension of the stomach, vagal excitation and gastrin (Kahlson *et al.*, 1964 ; Kahlson *et al.*, 1967). It should be noted that for a marked effect of nicotine on the histamine metabolism in the rat, feeding is essential. High, Shepherd & Woodcock (1965) have shown that surgical removal of the whole stomach in the female rat is followed by a fall in the urinary excretion of free histamine to considerably less than half the normal value. Among the organs investigated, the stomach possesses the highest ability to form histamine in the non-pregnant female rat (Kahlson *et al.*, 1963). The possibility that nicotine may release

histamine must also be borne in mind because mobilization of preformed mucosal histamine is known to incite a long lasting increase in mucosal histidine decarboxylase activity (Kahlson *et al.*, 1964).

Westfall (1965) found that the subcutaneous injection of nicotine caused a significant increase in the 24 hr urinary excretion of adrenaline but not of noradrenaline in the rat. Nicotine and histamine release catecholamines from the adrenal glands (Dale & Laidlaw, 1912; Feldberg, 1941; Vogt, 1951; Staszewska-Barczak & Vane, 1965) and catecholamines have been shown to cause changes in histamine metabolism—for example, a lowering of HFC in rat lung (Graham *et al.*, 1964). It remains for further work to establish whether a liberation of catecholamines plays a part in the changes in histamine metabolism reported in the present study.

SUMMARY

1. The effect of nicotine on the histamine metabolism in the rat has been studied *in vivo* and *in vitro*.

2. Nicotine greatly enhances the rate of histamine formation in the gastric mucosa, but not in the lung or skin.

3. The elevated mucosal histamine forming capacity is paralleled by an increased urinary excretion of histamine.

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