Inhibition of the taenia of the guinea-pig caecum by acetylcholine, nicotine or 5-hydroxytryptamine

SIR,—During an analysis of the mechanisms of the contractile responses of the taenia induced by drugs, it was observed that in the presence of hyoscine (0·1 μg/ml), nicotine (1–10 μg/ml) produced a relaxation of the preparation. High doses of acetylcholine (10–20 μg/ml) or 5-hydroxytryptamine (5-HT) (10–20 μg/ml) induced a biphasic effect—a relaxation followed by a contraction. The size of these inhibitory responses varied greatly and was dependent on the tone of the preparation, and this tone often varied in the course of an experiment. No inhibitory responses were observed on preparations which did not exhibit tone. It was found that a preparation with a stable degree of tone could be obtained with histamine (0·2 μg/ml) included in the bath fluid. This concentration of histamine produced more than 80% of the maximal contraction of the taenia. In the presence of this artificial tone, inhibitory responses were recorded with nicotine (2–20 μg/ml), acetylcholine (20–100 μg/ml) and 5-HT (10–100 μg/ml). The mechanisms of these responses were investigated.

A 5 cm length of the taenia was set up in Krebs solution at 37° containing hyoscine (0·1 μg/ml) and histamine (0·2 μg/ml). The solution was gassed with a mixture of oxygen 95% and carbon dioxide 5%. The concentrations of the drugs are expressed as the base. The responses were recorded on a smoked drum by means of an isotonic side-writing balsa wood lever.

The inhibitory responses to nicotine or acetylcholine were blocked by hexamethonium (20–40 μg/ml), dimethylphenylpiperazinium (5 μg/ml) or pentolinium (5 μg/ml). The local anaesthetic agents, procaine (10 μg/ml) or cocaine (1–20 μg/ml), abolished the relaxations caused by acetylcholine and greatly reduced those produced by nicotine. Higher concentrations of these drugs reduced the artificially induced tone and thus made the interpretation of their effects on the responses difficult. However, when experiments were made with preparations which exhibited tone in the absence of histamine, 50 μg/ml of procaine blocked the relaxations to nicotine or to acetylcholine. The inhibitory responses to 5-HT were not modified by any of these agents.

Guanethidine (1–10 μg/ml) reduced the responses to nicotine, blocked those to acetylcholine, but did not antagonize the relaxation produced by 5-HT. The responses to acetylcholine or to nicotine returned as soon as the blocking drug was washed out of the bath. This is in contrast to the usual persistent adrenergic blockage produced by this compound (Maxwell, Plummer, Schneider, Povalski & Daniel, 1960).

It was found that noradrenaline or isoprenaline relaxed the taenia. The responses to isoprenaline, but not to noradrenaline, were eliminated by pronethalol (1–2 μg/ml). The responses to nicotine or acetylcholine were reduced by this β-adrenergic blocking drug, but the effect of 5-HT remained unchanged. Hydergine (a mixture of equal parts of dihydroergocornine, dihydroergocryptine and dihydroergocristine) at a concentration of 3 μg/ml almost abolished the inhibitory responses to noradrenaline but not those produced by isoprenaline. The inhibition induced by acetylcholine or nicotine but not that caused by 5-HT was reduced by hydergine. Phenoxybenzamine (1 μg/ml) or piperoxan (1–5 μg/ml) antagonized the histamine-induced tone.

Two guinea-pigs were pretreated with reserpine (10 mg/kg) 24 hr before the experiment and two others were given reserpine 0·5 mg/kg daily for 10 days. A daily dose of 15 mg/kg of guanethidine was given to two guinea-pigs for 3 days and the animals were killed on the fourth day. In all the experiments made with preparations from these animals there was no evidence that the pretreatment modified the responses.

These experiments may be summarized in Table 1.

TABLE 1. SUMMARY OF THE EFFECTS OF ANTAGONISTS ON THE INHIBITORY RESPONSES

Antagonist	Relaxation to:		
	Acetylcholine	Nicotine	5-Hydroxytryptamine
Ganglion blocking agent (hexamethonium or pentolinium)	blocked	blocked	no effect
Procaine or cocaine	blocked	blocked	no effect
Guanethidine	blocked (non-persistent) effect	Reduced (non-persistent) effect	no effect
Pronethalol or Hydergine	reduced	reduced	no effect
Pretreatment with reserpine or guanethidine	no effect	no effect	no effect

Weis (1962) reported that dimethylphenylpiperazinium relaxed the isolated taenia of the guinea-pig caecum, and attributed the response to either the stimulation of adrenergic nerves or to the release of catecholamines from chromaffin cells.

The result I now report with ganglion blocking drugs or local anaesthetic agents are consistent with the involvement of a nerve pathway in the responses to nicotine or acetylcholine. But the evidence is not wholly consistent with the view that these drugs stimulated adrenergic nerves. Thus, although guanethidine reduced the responses, the characteristic prolonged blockade (Maxwell & others, 1960) was not seen. In fact, the blocking effect resembled the ganglion blocking activity of guanethidine (Maxwell & others, 1960).

Reserpine or guanethidine pretreatment did not seem to modify the relaxations to nicotine of acetylcholine. Similar concentrations of guanethidine (Boyd, Gillespie & MacKenna, 1962) or reserpine (Bentley, 1962; Day & Rand, 1964) have been shown to reduce or abolish the responses of other intestinal preparations to sympathetic nerve stimulation. The result of these pretreatments also renders it unlikely that these drugs induced the responses by the release of catecholamines from (non-innervated) chromaffin cells.

The most acceptable interpretation of these experiments appears to be that nicotine or acetylcholine stimulated inhibitory nerves which did not release noradrenaline (or adrenaline) at their terminations. Burnstock, Campbell & Rand (1966) have also reached a similar conclusion that there may be inhibitory nerves distinct from adrenergic nerves.

The relaxations of the taenia produced by 5-HT were not modified by any of the antagonists used. It seems probable that this effect of 5-HT arose from a direct action on the smooth muscle cells.

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The role of dopamine in motor excitation of mice induced by brain catecholamine releasers

SIR,—Animals treated with monoamine oxidase inhibitors show intense behavioural excitation after the administration of drugs that release catecholamines in the brain. This excitation is currently interpreted as the effect of an excess of free and active amines reaching their receptors (Brodie, Pletscher & Shaw, 1956; Costa, Gessa, Kuntzman & Brodie, 1962; van Rossum & Hurkmans, 1963; Graeff, Garcia Leme & Rocha e Silva, 1965). However the specific role played by noradrenaline or dopamine in this phenomenon is still uncertain.

The experiments now presented suggest a predominant participation of dopamine in the psychomotor stimulation of mice after reserpine or α-methyl-*m*-tyrosine when given after a monoamine oxidase inhibitor.

Seventy male albino mice, 20–25 g, were divided into 7 groups for different drug treatments. In each experiment one pair of mice had its motor activity continuously registered during the 5 hr after the last drug injection by means of a photoelectric actometer (van Rossum & others, 1962). The treatment schedule and the results are summarized in Table 1. All drugs were dissolved in saline except reserpine (Serpasol, Ciba, Brazil) which was diluted in distilled water.

TABLE 1. INHIBITION BY α-METHYL-*m*-TYROSINE OF MOTOR STIMULATION CAUSED BY BRAIN CATECHOLAMINE RELEASE IN MICE

	Treatment (doses in mg/kg)	Activity counts*
I	Saline (i.p.) 15 min after MAOI†	3·92 ± 2·81
II	Reserpine (10, i.p.) 15 min after MAOI	60·26 ± 7·41
III	Methyltyrosine (160, i.v.) and after 5 hr, MAOI + reserpine (10, i.p.)	10·44 ± 7·14
IV	Methyltyrosine (160, i.v.) and after 24 hr, MAOI + reserpine (10, i.p.)	57·50 ± 3·71
V	Methyltyrosine (50, i.p. 15 min. after MAOI	38·98 ± 8·93
VI	Methyltyrosine (160, i.v.) and after 5 hr, MAOI + methyltyrosine (50, i.p.)	11·76 ± 4·78
VII	Methyltyrosine (160, i.v.) and after 24 hr. MAOI + methyltyrosine (50, i.p.)	36·72 ± 11·21

*Total number of impulses recorded during 100 min of maximal activity; figures represent the mean and standard error of 5 pairs of mice.
† Monoamine oxidase inhibitor: *N*-(1,4-Benzodioxan-2-yl)-*N*-benzylhydrazine tartrate (2596-IS, base-62%), 80 mg/kg i.p.

Five hr after the depleting dose of α-methyl-*m*-tyrosine (160 mg/kg, i.v.) there was a sharp reduction in the psychomotor stimulation induced by reserpine or methyltyrosine (50 mg/kg, i.p.) injected after monoamine oxidase inhibition; the response returned to control values 24 hr later. Data reported by Costa & others (1962) indicate that the dose of methyltyrosine employed (160 mg/kg, i.v.) gives an almost complete depletion of brain noradrenaline of several days' duration whilst dopamine is only transitorily decreased; the maximum dopamine depletion occurs around 4 hr after the injection and the normal concentration is almost restored 24 hr later.

Our results suggest that a normal dopamine store is the only requirement for the production of motor stimulation by catecholamine releasers; however, the