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Inhibitory activity of 8β-carbobenzyloxyaminomethyl-1,6-dimethyl-10α-ergoline towards stimulant effects by 5-hydroxytryptamine and amphetamine on liver fluke, *Fasciola hepatica, in vitro*

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The powerful and long lasting anti-5-hydroxytryptamine effects of 8β-carbobenzyloxyaminomethyl-1-6-dimethyl-10α-ergoline (MCE) have been tested on the spontaneous rhythmical activity of *Fasciola hepatica, in vitro*. The stimulant effects of both 5-HT and amphetamine on this preparation were totally blocked for at least 2 hr by 0·01 μg/ml of MCE. One mole of MCE counteracts the effects of about 2×10^4 mole of 5-HT or amphetamine. A few other drugs exert inhibitory activity on this preparation but at far higher doses. A comparison between the data obtained for MCE and for bromolysergic acid diethylamide (BOL) reveals that MCE is a more potent inhibitory drug than BOL and that, moreover, it is characterized by total lack of depressant or paralysant effects.

THAT 5-hydroxytryptamine is a mediator of nerve action in certain invertebrates was suggested by Welsh (1953). Mansour (1957) showed that 5-HT stimulated muscular contractions both in intact and in degangliated *Fasciola hepatica, in vitro*, and that bromolysergic acid diethylamide (BOL) antagonized the stimulant effects not only of 5-HT but also of lysergic acid diethylamide and amphetamine. Compounds such as BOL, harmine and yohimbine were therefore considered likely to depress motility in the intact liver fluke by combining with tryptamine receptors, thus blocking by competition the site of action of endogenously released transmitter.

The similarity between the effects of amphetamine and 5-HT and the blocking action exerted by BOL on these effects were considered to be evidence that these amines may act on the same receptors (Mansour, 1957). These assumptions led us to examine the effects on the liver fluke of 8β-carbobenzyloxyaminomethyl-1-,6-dimethyl-10α-ergoline (MCE), whose strong and long lasting antagonism to 5-HT was reported both *in vitro* (Beretta, Ferrini & Glässer, 1965) and *in vivo* (Beretta, Glässer & others, 1965). From the literature it was not clear whether the tonic contractions seen after the administration of 5-HT or amphetamine were caused directly or whether these were only indirectly derived through a primary release of other transmitter agents such as catecholamines. We report some experiments to investigate this problem.

Experimental

MATERIALS AND METHODS

Flukes were obtained from bile ducts of bovine livers within 1 hr of the death of the host. They were washed and subsequently placed in Ringer solution (NaCl 0·9; $CaCl_2$ 0·006; KCl 0·04; $NaHCO_3$ 0·05; glucose 0·05 g in 100 ml of distilled water at 37°). The flukes were

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attached to an isotonic lever under slight tension according to Chance & Mansour (1949), and tracings were recorded on a kymograph. Degangliated preparations were obtained by cutting off the head of the worm just below the ventral sucker. The stimulant drugs were added to the bath and left in contact with the preparations for 2–3 min and then removed by washing with fresh Ringer solution.

After recording the spasmogenic effect of a drug, a period of at least 20–25 min was allowed to elapse before the administration of another drug. Inhibitory drugs were administered similarly, but the time contact with the preparation was always 15 min, after which the drug was removed and a new stimulant compound was administered.

DRUGS

Amphetamine sulphate, 5-hydroxytryptamine creatinine sulphate, dihydroergotamine tartrate, phentolamine methansulphonate (CIBA), adrenaline hydrogen tartrate, propranolol hydrochloride (ICI) and 2-bromolysergic acid diethylamide were dissolved in distilled water. MCE was dissolved in warm distilled water with the aid of a slight excess of maleic acid.

Results

Both 5-HT and amphetamine stimulated the intact parasites and degangliated preparations. The threshold doses of 5-HT (as creatinine sulphate) and amphetamine sulphate were 1–10 μg/ml ($0{\cdot}25$–$2{\cdot}46 \times 10^{-5}$ M) and 5–10 μg/ml ($1{\cdot}35$–$2{\cdot}70 \times 10^{-5}$ M) respectively.

No latency was observed between the administration of the drug and the start of the tonic contraction. After washing, when the drug was removed, tone subsided but contractions of higher amplitude and of faster frequency than normal appeared. At this time the administration of another stimulant drug elicited only a slight effect or no effect at all. This was observed both with 5-HT administered after 5-HT or amphetamine and with amphetamine administered after amphetamine or 5-HT. A period of 20–25 min between two successive administrations of stimulants was necessary to restore the original sensitivity. Nevertheless tachyphylaxis to 5-HT was often persistent and when this occured the experiment was stopped. In experiments evaluating antagonists, 5-HT and amphetamine (agonists) were always administered at doses of 200 μg/ml; these were active in 75–80% of the experiments.

In Table 1 are summarized the molar concentrations of MCE, dihydroergotamine, phentolamine and propranolol which inhibited the stimulant effects of 5-HT and amphetamine. Also included are the results of some experiments we made using BOL and those reported by Mansour (1957) for the same compound. The inhibitory effects of dihydroergotamine, phentolamine and propranolol were short lasting (20–40 min) in spite of the high dosage, and a good recovery of the effects of the two agonists was obtained after the first or second addition of them to the bath after the antagonist.

TABLE 1. ANTAGONISM EXERTED BY BOL, MCE, DIHYDROERGOTAMINE (DHE), PHENTOLAMINE AND PROPRANOLOL AGAINST THE SPASMOGENIC EFFECTS OF 5-HYDROXYTRYPTAMINE AND AMPHETAMINE ON *Fasciola hepatica in vitro*

Stimulant drugs (molar conc.)	Antagonists (molar conc.)				
	BOL	MCE	DHE	Phentol-amine	Propranolol
5-Hydroxytryptamine creatinine-sulphate 5×10^{-4}	$0{\cdot}2 \times 10^{-6}$ $0{\cdot}5 \times 10^{-6}$ $1{\cdot}0 \times 10^{-6}$ (*)	$2{\cdot}4 \cdot 10^{-8}$	$3{\cdot}7 \times 10^{-5}$ $7{\cdot}5 \times 10^{-5}$	$2{\cdot}8 \times 10^{-4}$ $5{\cdot}7 \cdot 10^{-4}$	$6{\cdot}7 \cdot 10^{-4}$
Amphetamine sulphate 5×10^{-4}	$0{\cdot}2 \times 10^{-6}$ $0{\cdot}5 \times 10^{-6}$ $1{\cdot}0 \times 10^{-6}$ (*)	$2{\cdot}4 \cdot 10^{-8}$	$3{\cdot}7 \times 10^{-5}$ $7{\cdot}5 \times 10^{-5}$	$2{\cdot}8 \cdot 10^{-4}$ $5{\cdot}7 \cdot 10^{-4}$	$6{\cdot}7 \cdot 10^{-4}$

(*) Data reported by Mansour (1957).

We found the inhibitory effects of BOL to occur at doses a little lower than those described by Mansour (1957); removal of the drug from the organ bath promptly restored a good sensitivity of the preparations towards the two amines. BOL always exerted a depressant action on the spontaneous rhythmical motality of the worms just as was previously described by Mansour (1957). In contrast, MCE in low concentration counteracted the effects of 5-HT or amphetamine for a long period

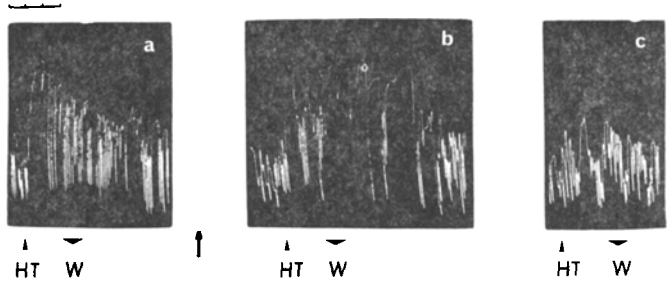

FIG. 1. Effects of 5-hydroxytryptamine creatinine sulphate (HT) 200 μg/ml administered before (a), 20 min (b) and 94 min (c) after 0·01 μg/ml of MCE at the arrow. The time contact of MCE was 15 min. At W washout. Time scale = 3 min.

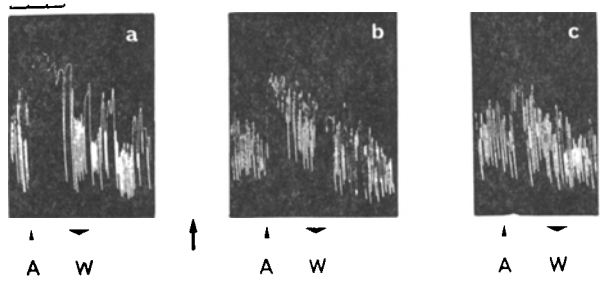

FIG. 2. Effects of amphetamine sulphate (A) 200 μg/ml administered before (a), 20 min (b) and 94 min (c) after 0·01 μg/ml of MCE at the arrow. The time contact of MCE was 15 min. At W washout. Time scale = 3 min.

(Figs 1 and 2). In some experiments 0·01 μg/ml of MCE caused an inhibition that could not be overcome for more than 2 hr. The first dose of 5-HT after antagonist did not elicit an inhibitory effect but on the contrary showed potentiation.

TABLE 2. THE RATIOS BETWEEN THE MOLAR CONCENTRATION OF THE ANTAGONISTS AND THE AGONISTS 5-HT AND AMPHETAMINE

BOL	MCE	DHE	Phentolamine	Propranolol
1 : 1000	1 : 20161	1 : 13·2	1 : 1·74	1 : 0·83
1 : 2500		1 : 6·6	1 : 0·87	
1 : 500 (*)				

(*) Ratio calculated on the base of the results of Mansour (1957).

MCE alone, at doses up to 1 μg/ml, did not affect the spontaneous motility of the liver fluke. No difference was found between the doses of antagonist needed to inhibit the effects of 5-HT or amphetamine. In Table 2 the ratios between the blocking molar concentrations of the inhibitory drugs and the molar concentrations of the stimulant drugs are summarized. The data in the first column have been calculated from both our results and those of Mansour (1957) with BOL, and it can be seen that one mole of MCE counteracts about 2×10^4 mole of 5-HT or amphetamine.

Discussion

The similarity of the effects elicited by 5-HT and amphetamine, and the evidence that these effects could be blocked by inhibitory drugs at the same dose level, suggests that the two amines probably act at the same receptor sites, which are peripheral and independent from the peri-ventosal ganglia. MCE proved to be the most powerful of the inhibitory drugs used, the ratio of its molar concentration to that of the agonists 5-HT and amphetamine being $1 : 2 \times 10^4$. The ratios for BOL were for 5-HT $1 : 1 \times 10^3$ and for amphetamine $1 : 2{\cdot}5 \times 10^3$ [the ratio calculated from the results of Mansour (1957) is $1 : 5 \times 10^2$]; for dihydroergotamine the respective figures were 1 : 13·2, 1 : 6·6; phentolamine 1 : 1·7, 1 : 0·9; propranolol 1 : 0·8.

These results strongly support the opinion that two amines act at a single tryptamine receptor (Mansour, 1957). It is noteworthy that at a dose of 0·01 μg/ml MCE strongly blocked the effects of 5-HT, whereas at a dose hundred times greater it is without effect in blocking the spontaneous movements of the fluke.

In this respect, MCE behaves quite differently from BOL which depresses spontaneous movements at doses 10 times smaller than those effective against 5-HT. The difference between BOL and MCE suggests that BOL is active at this site, or alternatively exerts a non-specific depression on the fluke, whereas MCE would appear to be active only against exogenous 5-HT and to be ineffective against endogenous transmitter. The last suggestion involves the conclusions of Welsh (1953) about 5-HT as a mediator of neuromuscular transmission in invertebrates.

The potentiation of the effects of the first dose of 5-HT after MCE, resembling the results obtained with this anti-5-HT compound on isolated rat uterus *in vitro* (Beretta, Ferrini & Glässer, 1965), may suggest action at true receptors and indicates that uptake on the receptors is slow since later on there is a long-lasting blockade.

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