ANTI-5HT ACTIVITY OF MEPHENESIN (MYANESIN) ON THE ISOLATED GUINEA-PIG ILEUM

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Summary: On the isolated guinea-pig ileum mephenesin had strong inhibitory effect against 5-HT (creatinine sulphate, 0.26 $\mu g/ml$ bath fluid) - the 50% inhibitory dose being 1.06 $\mu g/ml$. This effect was overcome by increasing the dose of 5-HT. Its 50% inhibitory doses against bradykinin (10 $\mu g/ml$), histamine (acid phosphate, 0.13 $\mu g/ml$) and barium chloride ($2H_20$, 66 $\mu g/ml$) were 4.6 μg, 56.6 μg and 5.3 μg per *ml* respectively. The inhibitory effect lasted much longer against 5-HT than against the other spasmogens. Cinchocaine a clinical spinal anaesthetic was used as control. Its 50% inhibitory doses against 5-HT, bradykinin, histamine, barium chloride and acetylcholine were 0.16, 2.93, 3.6, 1.66 and 7.33 μg per *ml* respectively. Dose-response lines of all agonists showed parallelism in the presence of mephenesin or cinchocaine. This suggests their common mechanism of inhibitory action.

It is suggested that the action of mephenesin on the central nervous system may be similar to its anti-5-HT action on the isolated guinea-pig ileum and through the alteration of the calcium carrier mechanism.

Key words: mephenesin cinchocaine 5-HT antagonism barium chloride bradykinin histamine

INTRODUCTION

Mephenesin (3-0-toloxy-1,2-propanediol) relaxes the tone of the voluntary muscle by acting on the central nervous system where it is known to prolong the synaptic recovery time (6) and reduce the inter-neurone repetitive discharge rate (7). The mechanism of action of mephenesin has been partly attributed to the stabilization of membrane potential by virtue of its local anaesthetic potency (5). Another mechanism of its action could be the competition with chemical mediators at the central receptors. On quantitative basis it is difficult to assess the antagonistic effect of mephenesin on the appropriate central nervous system receptors. Therefore the isolated guinea-pig ileum was used as a convenient model and cinchocaine, a typical spinal anaesthetic was used for comparison. 5-hydroxytryptamine, bradykinin, histamine, barium chloride and acetylcholine were used as agonists, some of which are neurohumors.

MATERIALS AND METHODS

Guinea-pigs of either sex (400-600 *g*) were stunned by a sharp blow on the head and bled. A 2-3 cm piece of ileum was cleaned and suspended in 15 *ml* bath containing Tyrode ($MgCl_2.6H_2O$ 100 *mg/l*) which was continously bubbled with oxygen and kept at 37°C. Sub-maximal doses of agonists, 5-hydroxytryptamine creatinine sulphate (0.26 $\mu g/ml$), synthetic bradykinin (BRS

640, very kindly supplied by Sandoz, Basle, Switzerland, 10 *ng/ml*) histamine acid phosphate (0.13 *µg/ml*), barium chloride ($BaCl_2.2H_20$, 66 *µg/ml*) and acetylcholoine hydrochloride (0.13 *µg/ml*) were used.

Mephenesin ampule (Myanesin, British Drug House) was a 10% W/V solution in 24% absolute alcohol and 0.1% propylene glycol. Suitable dilutions with Tyrode were made from the ampule. It was added to the bath 2 min before the addition of agonists. In 4 experiments, pure mephenesin powder dissolved in minimum quantity of alcohol and diluted with Tyrode, was used. For control, dilutions of 24% absolute alcohol corresponding to those used in mephenesin ampule were tested on the isolated guinea-pig ileum against 5-HT and acetylcholine.

Cinchocaine hydrochloride (Nupercaine ampulse, CIBA) containing 5 *mg/ml* in 6% dextrose solution was diluted with Tyrode and then added 2 min before the agonists. For control, 6% dextrose was tested on the guinea-pig ileum against 5-HT and acetylcholine. Mephenesin-induced and cinchocaine-induced percent inhibition of each agonist was plotted on semi-log paper.

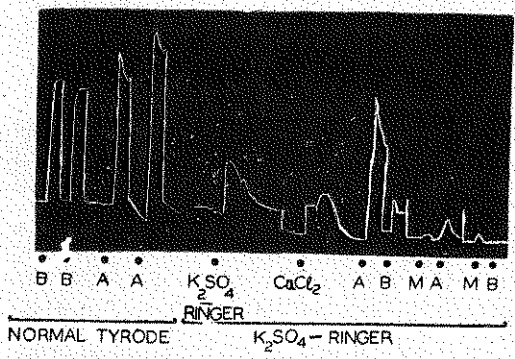

Fig. 1: Showing the effect of acetylcholine and 5-hydroxytryptamine on the isolated guineapig ileum in Tyrode solution and then in 'K_2SO_4 - Ringer'. Doses shown are per *ml* bath fluid. A, 1 *µg* acetylcholine hydrochloride; B, 2 *µg* 5-hydroxytryptamine creatinine sulphate; M, 5 *µg* mephenesin.

Experiments were also performed in Tyrode which had neither Na^+ nor Ca^{++}. For this purpose, the normal Tyrode was replaced by 'K_2SO_4 -Ringer' containing 17.13 *g* potassium sulphate, 417 *mg* potassium chloride, 360 *mg* potassium bicarbonate, 990 *mg* glucose, with distilled water to make 1 litre (4). The response of acetylycholine (0.66 *µg/ml*) and 5-HT (0.13 *µg/ml*) was first taken in the normal Tyrode. When the normal Tyrode was replaced by 'K_2-SO_4—Ringer', the tissue contracted and after about 3-5 min came back to the base line. Then the actions of calcium chloride (14 *µg/ml*), acetylcholine and 5-HT were tested. Finally the antagonistic activity of mephenesin (330 *ng/ml*) was tested against acetylcholine and 5-HT.

RESULTS

All doses have been expressed as per *ml* bath fluid. For each experiment 2-3 tissues have been used. Mephenesin and cincocaine blocked the stimulating effect of all the agonists

tested (Table I). The dose of mephenesin which induced 50% inhibition of 5-HT (creatinine sulphate, 0.26 μg), bradykinin (10 *ng*), histamine (acid phosphate, 0.13 μg), barium chloride ($2H_20$,66 μg) and acetylcholine (hydrochloride, 0.13 μg) were 1.06, 4.66, 56.6, 5.3 and 153 μg respectively. Alcohol (0.24%) in doses of 0.2 to 0.6 *ml*/15 *ml* bath fluid did not modify the response of acetylcholine and 5-HT. Both mephenesin powder and the ampuled solution showed inhibitory response against 5-HT.

TABLE I: Inhibitory activity of mephenesin and cinchocaine on the isolated guinea-pig ileum.

Agonist (*dose per ml bath fluid*)	*Dose* (μg/*ml bath fluid*) *which produced* 50% *inhibition*	
	Mephenesin	*Cinchocaine hydrochloride*
5-HT Creatinine sulphate (0.26 μg)	1.06	0.16
Bradykinin BRS 640 (10 μg)	4.6	2.93
Histamine acid phosphate (0.13 μg)	56.6	3.6
Barium chloride $.2H_20$ (66 μg)	5.3	1.66
Acetylcholine hydrochloride (0.13 μg)	153	7.33

The doses of cinchocaine which blocked 50% response of 5-HT creatinine sulphate, bradykinin, histamine acid phosphate, barium chloride and acetylcholine hydrochloride were 0.16, 2.93, 3.6, 1.66 and 7.33 μg respectively. Dextrose (6%) in dose of 0.1 to 0.5 *ml*/15 *ml* bath fluid did not modify the response of acetylcholine and 5-HT. Mephenesin and cinchocaine were found more potent against 5-HT than against bradykinin, hisamine, barium chloride or acetylcholine.

The inhibitory action produced by mephenesin or cinchocaine against the spasmogens was parallel which indicates a common mechanism of action.

When normal Tyrode was replaced with 'K_2SO_4-Ringer', the tissue contracted and then relaxed to the base line in 3-5 min. Calcium chloride ($CaCl_2.2H_20$, 14 μg/*ml*) added to the bath produced contraction which was sustained (Fig. 1). This effect of calcium chloride (14 μg) could be elicited for upto 70 min. After 30-40 min stay in 'K_2SO_4-Ringer' the action of acetylcholine decreased by 16% but of 5-HT by 70%.

DISCUSSION

Shah (8) observed that in the isolated guinea-pig ileum, 20.77 and 41.44 *mg*/*ml* of propylene glycol produced 34 and 94% inhibition respectively of the action of 5-HT (0.03-0.3 μg/*ml*). Mephenesin ampules used in this work contain 0.1% W/V propylene glycol which makes its final concentration 10 *ng*/*ml* in the bath fluid. Therefore, such a low concentration of propylene glycol in this work is not likely to inhibit the action of 5-HT and other spasmogens.

The present study on the isolated guinea-pig ileum shows that mephenesin and cinchocaine are more potent in blocking the action of 5-HT than of bradykinin, histamine, barium chloride

or acetylcholine. It further shows that the blocking effect of mephenesin and cinchocaine could be overcome by increasing the dose of 5-HT which indicate surmountable competitive type of the blockade. Also the inhibitory effect of mephenesin and cinchocaine lasted much longer against 5-HT than against the other spasmogens. In this connection it is interesting to note that 5-HT is considered as one of the transmitter substance in the central nervous system (1, 2). Therefore it is possible that the clinically important action of both these drugs on the spinal cord could be because of their anti-5-HT effect This point is further, though indirectly, supported by the fact that the anti-5-HT potency ratio and the local anaesthetic potency ratio of these two drugs are quite close-thus, cinchocaine (mol. wt. 347) is about 7 times more potent than mephenesin (mol. wt. 182) as an inhibitor of 5-HT (Table I) and is about 15 times more potent as a local anaesthetic (5).

5-HT acts on the calcium carrier mechanism of the excitable membrane (10) which affects the entry of calcium in the membrane. This action is generally considered to be responsible for smooth muscle contraction (3) brought about by 5-HT and perhaps for its central neurohumoral action. Therefore when immersed in Tyrode devoid of Na^+ and Ca^{++}, isolated tissues lose their sensitivity to 5-HT but not to acetylcholine because the latter acts by a different mechanism (4; present work). It is possible that mephenesin and cinchocaine bring about their central and peripheral actions by inhibiting the effects of 5-HT on the calcium carrier mechanism.

This allows a suggestion that the anti-5-HT activity as also the peripheral and central actions of mephenesin and cinchocaine are probably due to the stabilization of the membrane which, in turn, could be due to the alterations in the calcium carrier mechanism.

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