An independent nerve-pathway for 5-hydroxytryptamine in the guinea-pig ileum

SIR,—5-Hydroxytryptamine, like dimethylphenylpiperazinium or nicotine, contracts the longitudinal muscle of the guinea-pig ileum mainly by an action through a cholinergic nerve-pathway (Day & Vane, 1963). The evidence obtained from the selective use of autonomic blocking agents (Brownlee & Johnson, 1963) showed that 5-hydroxytryptamine activates receptors on the intramural parasympathetic ganglion cells which are pharmacologically distinct from the receptors activated by dimethylphenylpiperazinium or nicotine: the end result of this ganglion stimulation is the release of acetylcholine from the parasympathetic post-ganglionic nerve-terminals.

The question has been raised (Brownlee & Johnson, 1963) whether the specific ganglion receptors for 5-hydroxytryptamine are located on a cholinergic nerve-pathway which is separate from that stimulated by nicotine or dimethylphenylpiperazinium. Evidence in favour of this possibility has been obtained from experiments made to demonstrate the effect on the responses to drugs of the time course of inhibition of the cholinesterases of the guinea-pig isolated ileum by *NN*-diisopropylphosphorodiamidic fluoride (mipafox).

Harry (1962) and Johnson (1963a,b) based their choice of concentration of mipafox on the evidence obtained by J. Harry and G. D. H. Leach in this department in March, 1960. The figures they obtained for the inhibition of the esterases of the homogenised ileum of the guinea-pig by mipafox are reproduced below with their permission in Table 1. The relation between the concentration of mipafox and the inhibition per cent of the esterases whose substrate was butyrylcholine is given in Fig. 1 along with the relation between the mipafox concentration and the inhibition of the esterases whose substrate was acetylcholine, from which it is seen that butyrylcholinesterase is completely inhibited by 1×10^{-6} Molar and acetylcholinesterase by 1×10^{-5} Molar mipafox. With a molecular weight of 182 for the inhibitor the concentration for acetylcholinesterase inhibition becomes $1{\cdot}8 \times 10^{-6}$ g/ml. This figure of 1·8 μg/ml for the inhibition of all esterases of the homogenised guinea-pig ileum which hydrolysed acetylcholine provided the basis for the working concentration of 10 μg/ml of mipafox used in these experiments.

TABLE 1. INHIBITION OF ESTERASES OF HOMOGENISED ILEUM OF THE GUINEA-PIG BY MIPAFOX AT 37°C.

Manometer	Final molar conc.	−Log molar conc.	% Residual activity	% Inhibition
A	Control		100	0·0
B	$7{\cdot}5 \times 10^{-6}$	5·13	2·8	97·2
C	$7{\cdot}5 \times 10^{-7}$	6·13	31·96	68·04
D	$1{\cdot}5 \times 10^{-7}$	6·82	33·13	66·87
E	$7{\cdot}5 \times 10^{-8}$	7·13	44·72	55·28
F	$7{\cdot}5 \times 10^{-9}$	8·13	95·82	4·18
	Substrate Acetylcholine 0·0138M Total evolution of CO_2 from control manometer = 7,737 µl. CO_2/g/hr			
G	Control		100	
H	$7{\cdot}5 \times 10^{-6}$	5·13	2·01	97·99
J	$7{\cdot}5 \times 10^{-7}$	6·13	1·96	98·04
K	$7{\cdot}5 \times 10^{-8}$	7·13	23·15	76·85
L	$7{\cdot}5 \times 10^{-9}$	8·13	100·0	0·0
M	$7{\cdot}5 \times 10^{-10}$	9·13	100·0	0·0
	Substrate Butyrylcholine 0·0138M Total evolution of CO_2 from control manometer = 18,217 µl. CO_2/g/hr			

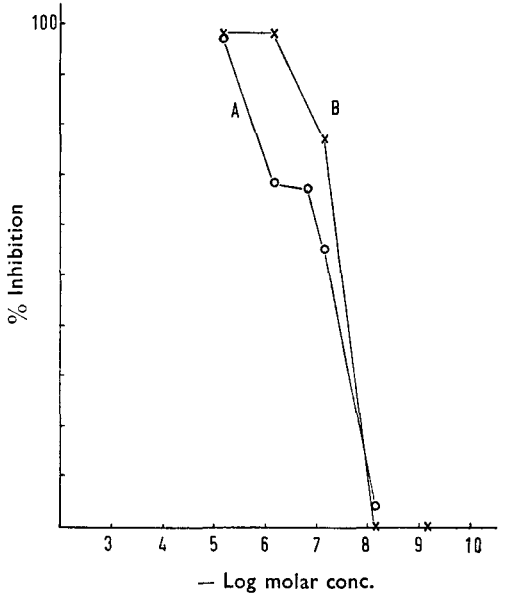

FIG. 1. The relation between the concentration of mipafox and the inhibition % of the acetylcholinesterase (A) and the butyrylcholinesterase (B) of the homogenised guinea-pig ileum (Warburg technique). Acetylcholine and butyrylcholine were each 0·0138 molar. Butyrylcholinesterase was completely inhibited by 1×10^{-6} molar (0·18 μg/ml) and acetylcholinesterase by 1×10^{-5} molar (1·8 μg/ml) mipafox (Harry, J. & Leach, G. D. H., unpublished results, 1960).

Treatment of the isolated ileum for 60 or 75 min with mipafox (10 μg/ml) potentiated maximally the responses to acetylcholine, 5-hydroxytryptamine and dimethylphenylpiperazinium but left the responses to histamine unaffected (Fig. 2). Further, the responses to 5-hydroxytryptamine were potentiated to a statistically significantly greater extent than either acetylcholine or dimethylphenylpiperazinium which, within the limits of the experimental error, were both potentiated to the same extent. The potentiation (measured as a displacement of the dose-response curve at a level of the E.D. 50 response) of the responses to 5-hydroxytryptamine was 0·927 $\pm$ 0·153 (log units $\pm$ s.e.), to dimethylphenylpiperazinium 0·486 $\pm$ 0·132 and to acetylcholine 0·375 $\pm$ 0·054. These potentiations in log units correspond to about eight-fold for 5-hydroxytryptamine, three-fold for dimethylphenylpiperazinium and two-fold for acetylcholine. Robertson (1954) also obtained similar results on the rabbit ileum treated with the acetylcholinesterase inhibitor 1,5-di(*p*-allyl-*N*-methylaminophenyl)-pentan-3-one dimethobromide.

After treatment with mipafox for only 1·5 min the responses to acetylcholine and dimethylphenylpiperazinium were potentiated, and this potentiation remained steady until after treatment for 40 min when the responses to these drugs were potentiated further. The 5-hydroxytryptamine responses were not potentiated by mipafox until after the 40 min treatment period when they were maximally potentiated. The responses to histamine were not significantly different from the control responses even after 80 min treatment with mipafox.

The differences in the degree of potentiation of 5-hydroxytryptamine on the one hand and dimethylphenylpiperazinium and acetylcholine on the other,

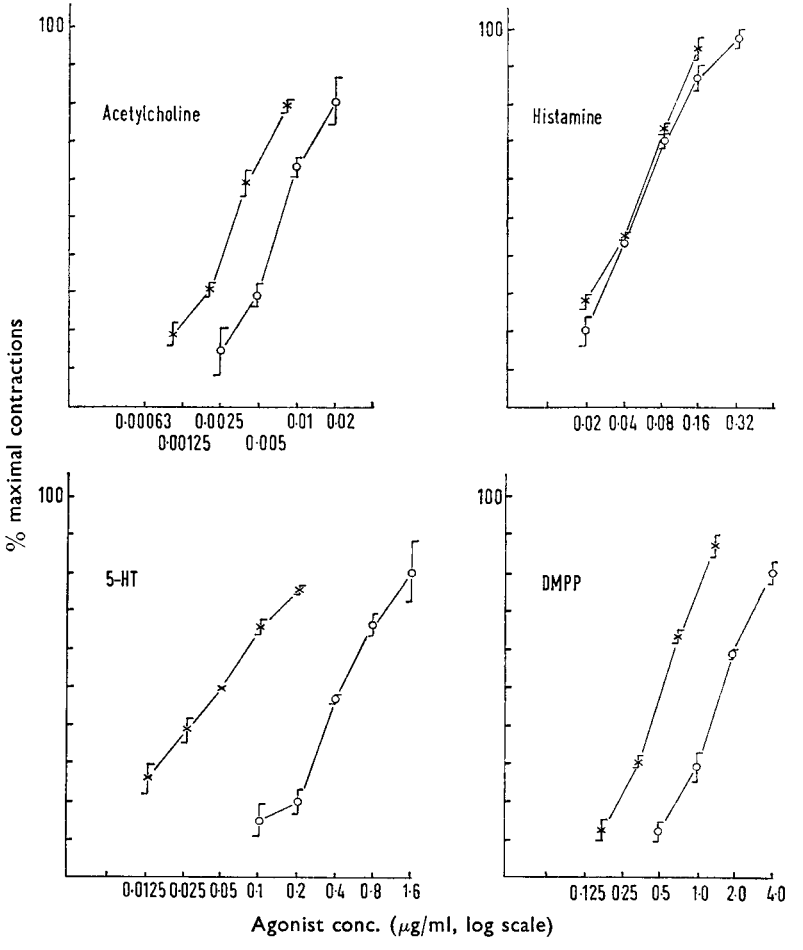

FIG. 2. The effect of complete inhibition of the cholinesterases of the guinea-pig ileum by treatment with mipafox (10 μg/ml) for 1 hr on its responses to acetylcholine, histamine, 5-hydroxytryptamine and dimethylphenylpiperazinium. ○ Responses to the agonists. × Responses after treatment with mipafox for 1 hr (s.e. is also shown). The curves for acetylcholine, 5-hydroxytryptamine and dimethylphenylpiperazinium were displaced to the left so that they were parallel to the originals. The histamine responses were unchanged. 5-Hydroxytryptamine was potentiated to a statistically significantly greater extent than either acetylcholine or dimethylphenylpiperazinium.

and the differences in the time course of inhibition of the cholinesterases on the responses of the ileum to drugs, are unexpected if dimethylphenylpiperazinium and 5-hydroxytryptamine are activating the same cholinergic nervous system, and furthermore these differences cannot be explained by the two drugs releasing different amounts of acetylcholine from the same nerve-endings since recent work in this department (Johnson, 1963, communicated to the British Pharmacological Society at Dublin) showed that equi-effective doses of the two drugs released equal amounts of acetylcholine; yet it was this acetylcholine which mipafox potentiated to a different degree when released by 5-hydroxytryptamine

from that released by dimethylphenylpiperazinium. In addition, the different time course of inhibition implied that the acetylcholine was liberated under different circumstances by the two drugs.

The known property of mipafox to discriminate between the two types of cholinesterase (Fig. 1.; Holmstedt, 1957) would favour the explanation that the acetylcholine released by 5-hydroxytryptamine is hydrolysed by a cholinesterase with different properties from that which hydrolyses the acetylcholine released by dimethylphenylpiperazinium or acetylcholine added exogenously, and it is difficult to imagine how this could arise unless 5-hydroxytryptamine is acting on a nerve-pathway independent from that activated by dimethylphenylpiperazinium. The greater potentiation of 5-hydroxytryptamine than dimethylpiperazinium by mipafox can be explained only by a difference in the amount of or nature of the cholinesterase at a separate nerve-ending.

The simplest explanation suggested by these experiments is that 5-hydroxytryptamine and dimethylphenylpiperazinium activate different nerve-pathways; the acetylcholine released by 5-hydroxytryptamine being hydrolysed mostly by acetylcholinesterase whereas that released by dimethylphenylpiperazinium, and also exogenous acetylcholine, being hydrolysed by a mixture of both butyryl and acetylcholinesterase.

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Structural consideration in the inhibition of rat brain acetylcholinesterase

SIR,—The presence of a negatively charged "anionic" site responsible for the binding of substituted ammonium ion, and an 'esteratic' site which binds the ester group is well established for acetylcholinesterase. Many quaternary ammonium compounds including neostigmine have been shown to be powerful inhibitors of this enzyme (Augustinsson & Nachmansohn, 1949). The preferential inhibition of true acetylcholinesterase by neostigmine without equally effecting other esterases (Wilson, Levine & Freiberger, 1952) and the presence of mainly true acetylcholinesterase in rat brain (Parmar, Sutter & Nickerson, 1961) led us to investigate the effect of tetramethylene, hexamethylene, octamethylene, decamethylene and dodecamethylene-bis(3-dimethylaminophenyl *N*-methylcarbamate) dimethobromides on acetylcholinesterase activity of rat brain homogenate, in order to show the role of the number of methylene groups connecting two neostigmine molecules present in these compounds.

Acetylcholinesterase activity in brain homogenate was estimated colorimetrically (McOsker & Daniel, 1959) using acetylthiocholine as the substrate