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

where hydrolysis resulted in thiocholine which was estimated by the nitroprusside reaction. Acetylcholinesterase activity of various subcellular fractions of rat brain determined colorimetrically with acetylthiocholine has been shown to be almost identical with the activity determined manometrically using acetylcholine or acetylthiocholine as the substrate (Parmar, Sutter & Nickerson, 1961). Inhibitory effects of neostigmine derivatives, at final concentration of 3×10^{-8}M, on acetylcholinesterase during hydrolysis of acetylthiocholine is shown in Table 1.

TABLE 1. INHIBITION OF ACETYLCHOLINESTERASE IN RAT BRAIN HOMOGENATE

Preincubation time Min	% inhibition					
	Neostigmine	$[CH_2]_4$*	$[CH_2]_6$*	$[CH_2]_8$*	$[CH_2]_{10}$*	$[CH_2]_{12}$*
0	0·0	0·0	3·9	7·7	16·0	27·0
	31·6	16·3	44·0	56·0	70·8	75·0
10	32·6 (31·9)	16·3 (15·9)	44·0 (44·3)	54·2 (55·9)	68·8 (69·0)	75·0 (74·2)
	31·6	15·0	45·0	57·5	67·5	72·5
20	61·0	65·4	71·6	79·3	92·0	100·0
30	71·6	73·1	73·5	80·7	100·0	100·0

* Bis (3-dimethylaminophenyl *N*-methylcarbamate) dimethobromide.
Enzyme activity was determined as change in extinction per 100 mg wet tissue during 10 min incubation. Each tissue sample was done in triplicate. The reaction mixture in a volume of 2 ml contained tris buffer (43·7 mM) pH 7·4, sodium chloride (350 mM), acetylthiochline ($1·5 \times 10^{-3}$M), 0·2 ml of 10% brain homogenate and inhibitors at the final concentration of 3×10^{-8}M. Suitable controls for substrate and tissue blanks were taken. The preincubation time denotes incubation of brain homogenate with the inhibitor for 10, 20 and 30 min before adding acetylthiocholine. Experiments with 0 time preincubation denote when both substrate and inhibitor were added at the same time. Figures in parentheses are averages.

Increase in the number of methylene groups in the compounds was found to increase enzyme inhibition. Inhibition also increased on preincubation of the enzyme preparation with these compounds for varying length of time before the addition of acetylthiocholine. Inhibitory effects of these derivatives could thus be modified by the substrate, indicating presumably the competitive nature of the inhibition. Our results are in good agreement with those reported earlier (Kraupp, Stumpf, Herzfeld & Pillat, 1955) where inhibition of dog serum and erythrocyte cholinesterase similarly increased with the length of the polymethylene chain of these compounds.

Our results indicate that true acetylcholinesterase possesses two 'anionic' sites to bind both quaternary ammonium groups of such bis-quaternary ammonium compounds (Bergmann, Wilson & Nachmansohn, 1950). The presence of the polymethylene chain in these derivatives may be assumed to facilitate such binding. Increase in the number of such methylene groups makes the compound more flexible which ultimately results in greater ease in binding to two "anionic" sites and thereby producing increased inhibition. Further studies may elucidate the exact role of such a polymethylene chain in the inhibition of brain acetylcholinesterase.

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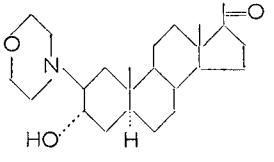
Anticonvulsant and interneuronal blocking activity in some synthetic amino-steroids

SIR,—The electroshock seizure threshold in animals can be raised or lowered by adrenocortical and sex hormones (Woolley & Timiras, 1962a,b and ref. cit.; Woodbury 1958 and ref. cit.). So far only limited success has been achieved in the search for synthetic steroids with potent central nervous system activity, yet devoid of hormonal actions, e.g. Brown & Sarett (1963). Certain amino-esters of 21-hydroxypregnanedione possess general anaesthetic activity (Figdor & others, 1957) and funtumidine (3α-amino-20α-hydroxy-5α-pregnane) is reported to cause tranquillisation (Blanpin & Quevauviller, 1960, and references cited).

We have investigated a series of amino-steroids for ability to produce loss of righting reflex. In addition, anti-tremorine activity, antagonism to electrically- and chemically-induced seizures and effects on blood pressure, neuromuscular transmission and the crossed extensor reflex in the cat have been examined.

The compounds tested were derivatives of androstane or pregnane in which the amino-radical (amino-, n-propylamino-, n-butylamino-, dimethyl-amino-, diethylamino-, piperidino- and morpholino-) was attached to C-2, C-6 or C-16.

Of the four related compounds, 3α-hydroxy-2β-morpholino-5α-pregnan-20-one (I), 3α-hydroxy-2β-morpholino-5α-pregnane-11, 20-dione (II), 3α-hydroxy-16α-methyl-2β-morpholino-5α-pregnan-20-one (III) and 3α-hydroxy-16α-methyl-2β-morpholino-5α-pregnane-11, 20-dione (IV), the least substituted (I), was the most potent in causing loss of righting reflex.



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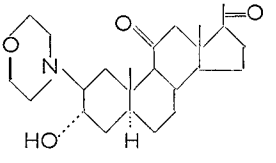

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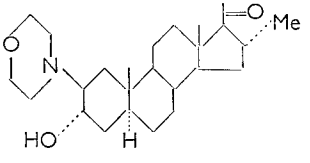
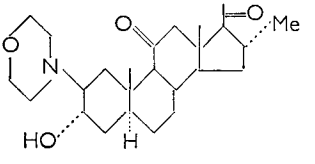


III



IV