PHARMACOLOGY

Effect of 'Tremorine' and some Anti-Parkinson's Disease Drugs on Acetylcholine in the Rat's Brain

Giarman and Pepeu[1,2] have demonstrated that atropine sulphate and scopolamine hydrobromide decrease total brain acetylcholine in the rat. Both drugs are effective against Parkinson's disease and prevent the tremor, rigidity and the parasympathetic stimulation induced by the administration of 'Tremorine' (1,4-dipyrrolidino-2-butyne)[3].

In the present work the effects of 'Tremorine' and of some anti-Parkinson's disease drugs on brain acetylcholine have been investigated. Adult male rats 150–200 g were killed by decapitation at the time stated in each experiment. The brain (excluding cerebellum, olfactory lobes and pituitary) was removed quickly and total acetylcholine was extracted by the method of Smallman *et al.*[4]. The acetylcholine content of the extracts was estimated on the isolated eserinized rectus abdominis muscle of the frog. The values for acetylcholine are expressed in terms of acetylcholine chloride. The effect of the various drugs used was carefully examined on the rectus abdominis in order to compensate for any influence on the response of the muscle to acetylcholine. All the drugs were administered to the rats intraperitoneally in 0·1–0·4 ml.

Table 1 shows that 'Tremorine' induces a marked rise of total brain acetylcholine which closely follows the onset of the symptoms. This finding is in agreement with the observation of Stern *et al.*[5] that 'Tremorine' stimulates brain cholineacetylase. Pretreatment of the rats with either atropine sulphate or caramiphen hydrochloride prevents the onset of the tremor, rigidity and parasympathetic stimulation induced by the injection of 'Tremorine' and completely prevents the increase of brain acetylcholine. 'Tremorine' has been shown to be a weak inhibitor of the cholinesterase of the erythrocytes and intestinal mucosa[6]. Atropine sulphate, on the other hand, does not prevent the increase of brain acetylcholine caused by the injection of eserine sulphate, which is presumably due to the inhibition of brain cholinesterases (Pepeu, unpublished work). Also diethazine, an anti-Parkinsonism drug of the phenothiazine type, prevents the onset of the symptoms caused by 'Tremorine'. Diethazine diminishes but not always abolishes the increase of brain acetylcholine due to 'Tremorine'. In fact 50 per cent of the rats in the group treated with diethazine and 'Tremorine' had a brain-level of acetylcholine higher than 3 μg/g. Nevertheless, all the rats had very little or no symptoms. Ahmed *et al.*[7] have shown that of the established anti-Parkinson's disease drugs diethazine is the least potent antagonist of acetylcholine on the isolated guinea pig ileum. Moreover, we have noted that caramiphen, like atropine, decreases brain acetylcholine but diethazine does not, as may be seen in Table 2. The rats injected with diethazine are slightly sedated whereas those injected with caramiphen show an increase of the spontaneous motility.

The results reported show a difference between the anti-Parkinson's disease drugs of the atropine type and those of the phenothiazine type. They do not give a definite evidence that the increase of brain acetylcholine is responsible for the symptoms induced by 'Tremorine'. Since 'Tremorine' also increases brain histamine in the rat[8] and depletes brain norepinephrine[9], the mechanism of action of this drug seems to be very complex and its action on brain acetylcholine only one of its aspects.

Table 2

Drugs	No. of animals	Dose (mg/kg)	Time (min.)	ACH (μgm/gm ± *S.E.*)	Remarks
None	26	—	—	2·80 ± 0·08	
Caramiphen hydrochloride	6	20	40	2·13 ± 0·14*	Mild excitation
Diethazine hydrochloride	6	40	40	2·85 ± 0·22	Sedation

* This value differs significantly from the controls at a level of $P < 0·02$

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Phenylquinone Writhing Test: Interpretation of Data

THE antagonism of phenylquinone (2-phenyl-1,4-benzoquinone)-induced writhing in mice has been recommended for investigating weak analgesic activity of new compounds[1,2], but the non-specificity of this test has been emphasized by Hendershot and Forsaith[3]. Many compounds not considered to be analgesics, for example, parasympathomimetics, sympathomimetics and central nervous system stimulants, protect mice against the writhing induced by phenylquinone. During the routine pharmacological screening of new compounds, we observed an apparent correlation between *in vitro* anti-5-hydroxytryptamine activity and the ability of a compound to antagonize phenylquinone-induced writhing. Therefore, to determine more precisely the value of this test in a routine screening programme, it was decided to investigate in more detail the writhing response and its modification by compounds and procedures known to affect the activity and metabolism of biogenic amines.

The *in vitro* anti-5-hydroxytryptamine activity of compounds was investigated using the isolated œstrus rat uterus preparation. Dose-response curves to 5-hydroxytryptamine (5HT) were obtained in the presence or absence of varying concentrations of antagonist and the concentration of antagonist required to reduce the sensi-

Table 1

Drugs	No. of animals	Dose (mg/kg)	Time (min)	ACH (μgm/gm ± *S.E.*)	Changes (%)	Remarks
None	26	—	—	2·80 ± 0·08	—	—
'Tremorine'	4	30	4–10	3·66 ± 0·74*	+ 30	Beginning of the symptoms
'Tremorine'	12	30	20–40	4·07 ± 0·64*	+ 45	Full symptomatology
Atropine plus 'Tremorine'	8	5 30	20 20	2·61 ± 0·20	—	No symptoms
Caramiphen plus 'Tremorine'	5	20 30	20 20	2·73 ± 0·32	—	No symptoms
Diethazine plus 'Termorine'	10	40 30	20 20	3·08 ± 0·40	+ 10	No or mild symptoms

*These values differ significantly from the controls at a level of $P < 0·001$