Antiacetylcholine activities of psychoactive drugs: a comparison of the [³H]quinuclidinyl benzilate binding assay with conventional methods

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The availability of specific radioactive affinity labels for muscarinic cholinoceptors, such as [³H]quinuclidinyl benzilate (³H-QNB, Yamamura & Snyder, 1974) or the aziridium ion from *N*-2'-chlorethyl-*N*-[2'',3'',³H₂]-propyl-2-aminoethyl-benzilate (Miller & Hiley, 1974), led to the introduction of simple and sensitive assays for the study of drug interactions with cholinoceptors. We have compared the antiacetylcholine potency of various drugs as determined by the ³H-QNB binding assay with data obtained from the more conventional tests, such as those based on the guinea-pig isolated ileum, on oxotremorine antagonism and on the effects on pupillary aperture.

³H-QNB (S.A. 1·52 Ci mmol⁻¹, 4·5 mCi mg⁻¹) was obtained from The Radiochemical Centre, Amersham. Rat brain homogenate (0·7 mg protein in 0·1 ml 0·32M sucrose) and the test compounds (10⁻³ to 10⁻⁷M in 0·2 ml) were incubated for 60 min at 27° with 4 pmol ³H-QNB in 2 ml 0·05M phosphate buffer pH 7·4. The incubation mixtures were filtered (Whatman GF/B) by suction, and the filters washed with ice-cold phosphate buffer and used for scintillation counting, as described by Yamamura & Snyder (1974). The IC50 is that concentration of drug which displaces specific ³H-QNB binding by 50%. Specific ³H-QNB binding is defined as the total binding minus the binding in the presence of 100 μmol oxotremorine (Yamamura & Snyder, 1974). In the oxotremorine test, the compounds were administered orally to groups of 10 mice 30 min before intraperitoneal application of oxotremorine (1 mg kg⁻¹). The ED50 is the dose which prevents the appearance of a strong tremor in 50% of the animals. Magnus's classical method was followed in assessing the spasmolytic action of the test drugs on guinea-pig isolated ileum. Acetylcholine was added to the organ bath at a concentration of 0·01 μg ml⁻¹. The IC90 is the concentration of the test drug in the organ bath (μg ml⁻¹) which reduces the acetylcholine-induced contraction by 90–100%. Pupillary aperture was measured with a binocular microscope (occular micrometer) in groups of 10 mice, under constant conditions of illumination, before and at fixed intervals after drug administration. The ED200 is the dose of the test drug which causes 100% increase in pupillary aperture.

As shown in Table 1, there is an excellent correlation between the effects of the various compounds in the ³H-QNB binding assay and the guinea-pig ileum (rs = 0·97; P < 0·001). This suggests that the properties

* Correspondence

of the cholinoceptors in the brain, which are investigated with the ³H-QNB assay, are similar to those of the cholinoceptors in the guinea-pig ileum. However, there is no correlation between the results of the *in vitro* assays and those of the oxotremorine test. For example, perlapine is active in the ³H-QNB binding assay and in the guinea-pig ileum preparation, but is completely inactive in the oxotremorine test. On the other hand, clothiapine is very effective against oxotremorine-induced tremors, its activity surpassing that of ditran and methixene and approaching that of atropine, but it is only weakly active in the ³H-QNB binding assay and the guinea-pig ileum. Thus, these activites are only 1/60 and 1/30 respectively of those of atropine. Likewise, there is no correlation between the effects on pupillary aperture and the actions in the ³H-QNB binding assay, the guinea-pig ileum and the oxotremorine tests.

The regulation of the pupillary aperture is effected peripherally by both cholinergic and adrenergic mechanisms, and it is not surprising, therefore, to find no correlation with the antiacetylcholine effects obtained with the other test systems. In the case of the centrally-

Table 1. *Antiacetylcholine activities of drugs as measured in various test systems.*

	³H-QNB assay IC50: μmol	G-pig ileum IC90: μg ml⁻¹	Oxot. antag. Oral. ED50: mg kg⁻¹	Pupil. apert. Oral. ED200 mg kg⁻¹
Ditran	0·008	0·01	7·4	1·3
Atropine SO₄	0·03	0·01	3·6	0·55
Methixene	0·06	0·02	8·8	7·5
Clozapine	0·30	0·16	9·0	4·4
Amitriptyline	0·30	0·30	9·5	11·0
Thioridazine	0·50	0·16	24·0	>300·0
Perlapine	1·20	0·16	inact.	27·0
Clothiapine	1·80	0·32	5·0	36·0
Chlorpromazine	2·6	0·40	17·0	>40·0
Loxapine	3·0	0·63	16·0	>40·0
Desipramine	4·4	1·26	inact.	9·00
Dibenzepin	10·0	10·0	inact.	27·0
Haloperidol	36·0	5·0	inact.	inact.

mediated oxotremorine tremor, antagonism depends not only on the antiacetylcholine activity of a compound, but on its ability to penetrate the blood-brain barrier and on its lipid solubility. Again, a correlation with the other tests is hardly to be expected. The fact that

clothiapine is more active in the oxotremorine test than would be expected from the results of the *in vitro* tests raises the question as to whether antagonism of oxotremorine-induced tremor depends exclusively on antiacetylcholine activity, or whether additional factors may be involved.

It is concluded that the ^{3}H-QNB binding assay and the guinea-pig ileum are equally useful for measuring antiacetylcholine activity *in vitro*. However, neither test permits reliable conclusions to be drawn concerning *in vivo* acetylcholine effects in the brain.

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Noradrenergic influence on the stereotyped behaviour induced by amphetamine, phenethylamine and apomorphine

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Many experiments indicate that stereotyped behaviour, consisting of sniffing, licking or biting, elucidated by amphetamines and apomorphine, is absolutely dependent on dopamine transmission in the basal ganglia of the mammalian brain (Randrup & Munkvad, 1974, review). Due to the close clinical similarity between the 'amphetamine psychosis' and certain forms of schizophrenia, the detailed study of amphetamine mechanisms and stereotyped behaviour has aroused great interest. It has been suggested that a cholinergic-dopaminergic balance in the brain is important in controlling the intensity of stereotyped behaviour (Arnfred & Randrup, 1968) and this concept has been of value in explaining the modifying influence of cholinergic systems on the primarily dopamine-dependent production of stereotyped behaviour in response to amphetamines. The concept of neuro-transmitter balance has also been of value in analysing the effects of pharmacological agents in some human extrapyramidal disorders, including Parkinsonism (Klawans, 1968).

We do not question the importance of dopamine transmission for stereotyped behaviour or its possible role in the pathogenesis of schizophrenia. We suggest, however, that the qualitative expression of stereotyped behaviour in different behavioural elements, here more specifically as a transition between sniffing, gnawing and licking, is also influenced by central noradrenaline transmission.

All experiments were in male Wistar rats, 200-250 g, housed in individual wire mesh cages (21 × 27 × 16 cm) at 21–23° with free access to food and water except in observation periods when food and water were withdrawn. Each rat was only used once. Apomorphine HCl (0·5 mg kg^{-1}, s.c.), (+)-amphetamine sulphate (5 mg kg^{-1}, s.c.) or β-phenethylamine hydrochloride (40 mg kg^{-1}, s.c.) in saline was injected at the start of the observation period and the rats were observed for the whole period of continuous sniffing induced by these three drugs. The numbers of rats showing occasional or continuous gnawing/biting at the bars (for apomorphine and amphetamine) or gnawing/licking at the bars (phenethylamine) within the stereotypy period, were recorded and included in the Tables. Gnawing is considered as compulsory biting. The whole-brain contents of homovanillic acid and dihydroxyphenyl acetic acid were estimated by the gas chromatographic technique of Braestrup, Andersen & Randrup (1975) and total MOPEG (3-methoxy-4-hydroxyphenylglycol) according to Braestrup (1973).

In our first series of experiments we investigated the ability of drugs with effects on central noradrenaline mechanisms to change the amphetamine or apomorphine-induced stereotyped sniffing into gnawing or biting. The results in Table 1 show that the noradrenaline receptor blocking drug, phenoxybenzamine, the inhibitor of noradrenaline synthesis diethyldithiocarbamate (DDC), which also reduces amphetamine metabolism (Jonsson & Lewander, 1973), and reserpine, which depletes noradrenaline (and dopamine) and markedly reduces its synthesis (Bræstrup & Nielsen, 1975), induce gnawing or biting in apomorphine- or amphetamine-treated rats. Administration of apomorphine or amphetamine alone in these small doses produced only sniffing behaviour and very infrequent licking. The drug clonidine, like phenoxybenzamine and DDC, induced gnawing or biting in amphetamine- or apomorphine-treated rats. Several experiments indicate that clonidine can inhibit central noradrenaline mechanism (Braestrup & Nielsen, 1976), and the lack of antagonism of the clonidine-induced gnawing by phenoxybenzamine (rather an intensification was noted) further supports the classification of clonidine as an inhibitor of at least one population of central noradrenaline neurons, though by a mechanism different

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