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Drug effect on acetylcholine level in discrete brain regions of rats killed by microwave irradiation

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Stavinoha, Peplke & Smith (1970) proposed the use of microwave irradiation for killing small laboratory animals and inducing a rapid and complete heat inactivation of the enzymes involved in acetylcholine (ACh) metabolism. Microwave irradiated brains can also be easily dissected into discrete areas. Relatively few papers are devoted to studies of drug effect on discrete brain areas compared with the large number of investigations carried out on the whole brain (Pepeu & Nistri, 1973).

removed, four areas were dissected out, ACh extracted in an acid medium and quantified by bioassay on the frog rectus abdominis. Choline acetyltransferase (ChAc) was determined by the radiochemical method of Fonnum (1969) in the same areas of a group of control rats killed by decapitation.

The ACh levels are reported in Table 1. They are similar to those reported by Schmidt, Speth, Welsch & Schmidt (1972) by gas chromatographic determination. These results confirm that irradiation gives higher ACh recovery than any previous method of sacrifice.

At the dose used each drug induced a non-uniform change in ACh content. In the frontal cortex which showed the lowest ChAc activity (2.19 ± 0.34 μmol $g^{-1} h^{-1}$) there was no ACh accumulation during pentobarbital anaesthesia but the highest ACh decrease during leptazol convulsions and hyoscine amnesia. The latter drugs

Table 1 Effect of some drugs on ACh (nmol/g ± s.e. mean) levels in discrete brain areas

Drug	*Dose (mg/kg i.p.)*	*Frontal cortex*	*Hippocampus*	*Caudate nucleus*	*Midbrain*
Saline (9)	–	22.78± 2.03	24.49± 1.32	56.30± 0.003	27.46± 1.92
Leptazol (4)	75	8.80±* 2.14	19.15± 2.75	25.53±* 2.09	18.98±** 0.93
Hyoscine (4)	0.5	10.01±* 1.76	18.61± 3.35	42.10±** 4.24	26.19± 4.45
Pentobarbital (4)	35	20.47± 2.42	35.55±** 5.28	78.65±* 3.19	31.86± 2.91

Comparison with saline treated rats: Student t test: * $P < 0.01$; ** $P < 0.02$.

In the present study we have examined the changes in ACh level induced in four different brain areas by some drugs which affect ACh level in the whole brain (Giarman & Pepeu, 1962).

A commercial unit, adapted by Medical Engineering Consultants (Lexington, Mass.), emitting a microwave output of 1350 watts was used for irradiating adult male Wistar rats. Irradiation time was 5 seconds. The brain was then

caused smaller variations in the hippocampus (3.89 ± 0.34 μmol $g^{-1} h^{-1}$) and midbrain (2.92 ± 0.34 μmol $g^{-1} h^{-1}$). In the caudate nucleus a 50% decrease in ACh content after leptazol was found in spite of high ChAc activity (7.62 ± 0.44 μmol $g^{-1} h^{-1}$).

In conclusion the drug induced changes in ACh content in discrete brain areas are not strictly related to the differences in ChAc activity.

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Interaction of neuroleptic and cholinergic drugs with central dopaminergic mechanisms

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Neuroleptic drugs have been shown to possess antidopaminergic and antimuscarinic properties (Miller & Hiley, 1974) using *in vitro* assay techniques. Animal behavioural models have now been used in order to examine the relative effects of these actions *in vivo*.

In rats with unilateral lesions of the nigro-striatal dopamine fibres induced by injection of 6-OH dopamine into the substantia nigra methamphetamine causes turning towards the side of the lesion in a dose-dependent fashion. Measurement of this turning behaviour is believed to constitute an *in vivo* measure of the effects of dopamine released from the nigro-striatal pathway on the intact side.

Turning produced by methamphetamine (5 mg/kg) was completely antagonized by the dopamine antagonists pimozide (0.25 mg/kg), α-flupenthixol (1.0 mg/kg) and α-clopenthixol (8 mg/kg). It was partially antagonized by α-flupenthixol (0.2 mg/kg) or chlorpromazine (4 mg/kg). The isomeric trans forms of the thioxanthenes β-flupenthixol (10 mg/kg) or β-clopenthixol (8 mg/kg) were ineffective. These trans isomers are also ineffective in blocking the stimulating effects of dopamine on striatal adenylate cyclase, whereas the α-(*cis*)-isomers are potent blockers. The dopamine antagonists clozapine and thioridazine in doses up to 18 mg/kg had no effect on turning. These are the two neuroleptics which combine antidopaminergic actions with potent antimuscarinic properties (Miller & Hiley, 1974).

The effect of cholinergic drugs on turning behaviour was also examined. Oxotremorine (0.75 mg/kg) plus methylatropine (5 mg/kg) antagonized methamphetamine induced turning, indicating the dopaminergic/cholinergic balance modulating the action of the extrapyramidal system. In addition, scopolamine (10 mg/kg) caused turning in the same direction as amphetamine but was less effective. Pimozide (0.25 mg/kg) or α-flupenthixol (0.2 mg/kg) antagonized the effects of scopolamine.

Low doses of apomorphine (<0.1 mg/kg) produced turning away from the side of lesion, presumably due to a direct action on supersensitive receptors on the lesioned side. This turning was not antagonized by thioridazine or clozapine (18 mg/kg). Apomorphine induced turning was antagonized by oxotremorine (0.74 mg/kg). These results indicate that the effects of cholinergic drugs are not wholly dependent on the integrity of the nigro-striatal system.

d-Amphetamine (4 mg/kg) produced a stimulation of locomotor activity in adult and 11-day old rats. Pretreatment of adults with clozapine (4 mg/kg) or thioridazine (4 mg/kg) 3 h previously or thioridazine (4 mg/kg) 30 min previously had no effect on the amphetamine induced stimulation of locomotor activity. Trifluoperazine (0.4 mg/kg) 3 h previously completely inhibited locomotor activity. Pretreatment of 11-day old rats with 4 mg/kg clozapine or thioridazine 3 h before amphetamine completely inhibited the stimulation of locomotor activity.

It is known that 11-day old rats can respond to catecholaminergic agonists but the response to cholinergic drugs has not yet developed. It is suggested that in adult animals the anticatecholaminergic effects of some neuroleptics may be