Further evidence for an interaction of propranolol with the central 5-hydroxytryptamine (5-HT) receptor

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There is accumulating evidence that propranolol and related β-adrenoceptor antagonists may have 5-HT receptor antagonist activity in addition to their well known β-receptor and membrane stabilising activity. The evidence derives mainly from experiments *in vitro* (Schecter & Weinstock, 1974), the blockade of behavioural syndromes in rodents thought to be 5-HT mediated (Green & Grahame-Smith, 1976; Weinstock, Weiss & Gitter, 1977) and from the measurement of the displacement of [^{3}H]-5-HT from its receptor on CNS derived synaptic membranes of the rat (Middlemiss, Blakeborough & Leather, 1977). The present studies investigate the interaction of β-adrenoceptor antagonists with the [^{3}H]-LSD receptor and report *ex vivo* evidence for antagonism of the central [^{3}H]-5-HT and [^{3}H]-DHA receptors by (−)-propranolol dosed parenterally.

Synaptic membranes from whole rat brain (minus the cerebellum) were prepared and binding studies carried out as previously described (Middlemiss *et al.*, 1977). Tritiated ligand concentrations were as follows: [^{3}H]-5-HT (5 nM); [^{3}H]-dihydroalprenolol (DHA 1 nM), [^{3}H]-LSD (2 nM). Non specific binding was defined as the radioligand bound in the presence of a large excess of cold drug: 5-HT (1 μM), (−)-propranolol (0.1 μM) and LSD (1 μM), respectively.

The displacement of [^{3}H]-LSD was, as has previously been shown for the displacement of [^{3}H]-5-HT, stereospecific with (−)-being more potent than (+)-propranolol. The potency of β-adrenoceptor antagonists against [^{3}H]-5-HT binding was equal to or greater than their potency against [^{3}H]-LSD binding. In contrast, classical 5-HT antagonists have a considerably greater affinity for [^{3}H]-LSD binding sites (Table 1). Our findings with classical 5-HT agonists and antagonists are in general accord with the published data of Bennett & Snyder (1976). Thus β-adrenoceptor antagonists also displace [^{3}H]-LSD from its receptor as would be expected if this class of drug possesses anti-serotonergic activity.

In order to demonstrate that the interaction with the 5-HT receptor may occur *in vivo*, propranolol was administered to rats, and one hour later a standard synaptosomal preparation made. (−)-Propranolol (10 mg/kg i.p.) but not (+)-propranolol (40 mg/kg i.p.) caused an apparent reduction in the number of [^{3}H]-5-HT receptors. Similar results were obtained with (−)-propranolol (3 mg/kg i.p.) against the [^{3}H]-DHA receptor. (+)-propranolol was inactive at 10 mg/kg i.p.

Table 1 Potencies of Drugs on [^{3}H]-5HT and [^{3}H]-LSD binding assays

Drug	IC_{50} (nM) [^{3}H]-5-HT	IC_{50} (nM) [^{3}H]-LSD	IC_{50} (5-HT)/IC_{50} (LSD)
Oxprenolol	430	6300	0.068
Alprenolol	450	3200	0.14
(−)-Propranolol	350	910	0.38
(+)-Propranolol	11,000	9400	1.2
Practolol	$>10^5$	$>10^5$	—
5-Hydroxytryptamine	17	80	0.21
Tryptamine	550	2100	0.26
5-Methoxytryptamine	92	270	0.34
5-MeODMT*	164	260	0.63
5-HODMT*	120	180	0.72
LSD	24	7.8	3.1
Cinanserin	3600	990	3.6
Methiothepin	430	56	7.7
Mianserin	4900	610	8.0
Cyproheptadine	4700	390	12
Methysergide	280	23	12

* 5-MeODMT: 5-methoxy-*N*,*N*-dimethyltryptamine.
* 5-HODMT: 5-hydroxy-*N*,*N*-dimethyltryptamine.
IC_{50}'s mean of at least two separate experiments.

These results lend support to the hypothesis that propranolol may be a central 5-HT antagonist in animals.

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Behavioural and biochemical studies in rats with N-methyl-D-aspartic acid and kainic acid

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Both kainic acid and N-methyl-DL-aspartate are excitatory amino acids believed to act at receptors for acidic amino acids. Of these two, kainate is believed to be excitotoxic at glutamate receptors (Olney, Rhee & Ho, 1974) and is widely used as a neurotoxic tool to produce models of neurological disorders associated with nervous degeneration, such as Huntington's chorea (Coyle, Schwarcz, Bennett & Campochiaro, 1977). N-Methyl-DL-aspartate is also a potent excitatory amino acid with some reported toxic properties (Olney, 1978). However, the active form of this compound is likely to be the D-isomer (Curtis & Watkins, 1963; Johnston, Curtis, Davies & McCulloch, 1974), and we have therefore explored the possible behavioural and neurotoxic properties of N-methyl-D-aspartate (NMDA) in comparison with those of kainic acid following acute and chronic injection into the rat globus pallidus.

Bilateral injections of NMDA (12 and 30 nm), kainic acid (2.3 nm) or vehicle (1.5 μl 0.05 M phosphate buffer, pH 7.2) were made stereotaxically into the globus pallidus of male Porton rats (A + 6.5, L ± 2.5, V − 1.0).

Animals were allowed to recover from surgery and behavioural and biochemical analyses conducted at 10 days. In a further series of animals, bilateral guide cannulae were mounted in the skull above the globus pallidus.

Acute bilateral injection of both NMDA and kainic acid (range 0.2–4 nm) induced a dose-dependent stimulation of motor activity. The lower doses of both amino acids produced sniffing and a mild locomotor response which was active for up to an hour ($P < 0.05$ for line crossing in an open field in comparison with vehicle-injected controls). The higher doses of NMDA and kainic acid produced various forms of hyperactivity and dyskinetic reactions including posturing, teeth grinding and torticollis. These behaviours were interposed between bursts of running seizure.

Chronically, both kainic acid and NMDA injected animals showed increased locomotor activity, with greater incidences of line crossing and rearing in the perimeter of an open field ($P < 0.01$ compared to vehicle-injected controls). Whereas acutely both amino acids exhibited similar potency for inducing hyperactivity, chronically 5–10 times the injected dose of NMDA was required to give the equivalent behavioural response as kainic acid.

Biochemically kainic acid (2.3 nm) induced a spectrum of changes within the injection site including reduced glutamate decarboxylase (GAD) (33% of control, $P < 0.001$) and choline acetyltransferase (ChAT) activity (55% of control, $P < 0.01$) and decreased concentrations of GABA and glutamate (39% of control, $P < 0.01$). NMDA (12 and 30 nm) caused a significant increase in glutamate concentrations ($P < 0.01$) and a reduction in ChAT activity ($P < 0.01$). The higher dose of NMDA caused a small, but significant decrease in pallidal GAD activity ($P < 0.05$).

Whilst the behavioural effects of these two excitant amino acids were similar, the biochemical profiles differed. Kainic acid is an agonist at glutamate receptors, while NMDA may be selective for aspartate receptors (Watkins, 1978). These two agents may provide useful tools to distinguish between the roles of glutamate and aspartate receptors within the central nervous system.