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Comparisons between phencyclidine, its monohydroxylated metabolites, and the stereoisomers of *N*-allyl-*N*-normetazocine (SKF 10047) as inhibitors of the muscarinic receptor and acetylcholinesterase

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Phencyclidine (PCP) is an arylcycloalkylamine derivative structurally and pharmacologically similar to the dissociative anaesthetic, ketamine, and is currently an illicit drug of abuse in the United States. Interest in the biochemical and physiological mechanisms by which this drug acts is presently high because of its psychotomimetic properties in man (Luby et al 1959; Davies & Beech 1960) and because of its recently realized abuse potential. Although considerable data suggest that PCP is an indirect dopaminergic agonist similar in some respects to methylphenidate (Garey & Heath 1976; Doherty et al 1980; Fessler et al 1980: Johnson & Oeffinger 1981), PCP is also known to possess anticholinesterase and antimuscarinic activity with the antimuscarinic potency being ten to twenty fold greater than the anticholinesterase potency (Kloog et al 1977; Aronstam et al 1980). Nevertheless, we have recently reported additive interactions between oxotremorine, a muscarinic agonist, and PCP suggestive of a prominent cholinomimetic action of PCP in mice (Johnson & Wilkenfeld 1981). Other authors have noted cholinomimetic properties of PCP in cross-tolerance studies in mice (Pinchasi et al 1978) and in cultured cardiac cells (Fosset et al 1979). To elucidate the behavioural relevance of these two mechanisms, we sought to determine the relative potency of PCP and other drugs known to produce PCP-like behavioural effects as potential inhibitors of both acetylcholinesterase and of the brain muscarinic receptor.

To this end we used two monohydroxylated metabolites of PCP which, while not without activity, are much less potent than PCP (Shannon 1981). We also tested *N*-allyl-*N*-normetazocine (SKF 10047), a benzomorphan which produces behavioural patterns similar to those of PCP (Martin et al 1976; Vaupel & Jasinski 1979; Holtzman 1980). SKF 10047 is particularly useful because its (+)-isomer, but not its (−)-isomer, has been shown to produce PCP-appropriate responding in both rats and monkeys using the drug discrimination paradigm (Brady et al 1981).

Anticholinesterase activity was measured according to the colorimetric procedure of Ellman (1961) using bovine erythrocyte acetylcholinesterase (Sigma Chemical Co.). The reaction mixture consisted of 0·1 M phosphate buffer (pH 7·4), acetylthiocholine bromide (0·125 mM), 5,5′-dithiobisnitrobenzoic acid (0·01 mM), test compounds (from 0 to 0·5 mM), and the enzyme in a total volume of 1·0 ml. The reaction was carried out in disposable cuvettes as we found that standard washing procedures did not adequately remove the SKF 10047 compounds from quartz cuvettes. The rate of absorbance change was monitored at 412 nm. The rate of non-enzymatic hydrolysis, determined in an enzyme-free control reaction, was subtracted from the experimental rate. The IC50 value of each inhibitor was calculated from a plot of fractional inhibition vs log drug concentration by computer fitting the data to the logistic function using the method of DeLean et al (1978).

Muscarinic receptor antagonism was estimated by measuring the specific binding of [^{3}H]quinuclidinyl benzilate ([^{3}H]QNB) to a preparation of rat cortical membranes according to Yamamura & Snyder (1974). Approximately 300 μg of membrane protein was incubated in a total volume of 2·0 ml containing 50 mM sodium, potassium phosphate buffer (pH 7·4) and [^{3}H]QNB (200 pM) in the presence or absence of the test drug for 60 min at 37 °C. Bound[^{3}H]QNB was trapped on Whatman GF/B glass fibre filters, and washed twice with 5 ml of buffer. The filters were air dried and counted after shaking for 30 min in Aquasol 2. Specific binding was defined as total binding less that in the presence of 10 μM atropine sulphate. IC50 values were obtained as described above.

We observed that PCP weakly inhibited acetylcholinesterase and that neither 4-phenyl, 4-piperdinocyclohexanol HCl (4-OH-cyclo PCP) nor 1-(1-phenylcyclohexyl)-4-hydroxypiperidine HCl (4-OH-pip PCP) inhibited

Table 1. Effect of PCP, its monoxydroxylated metabolites, and the stereoisomers of SKF 10047 on (I) acetylcholinesterase, (II) on the specific binding of [^{3}H]QNB to a membrane homogenate prepared from rat cerebral cortex.

Drug	IC50 ± s.e.* (μM)	
	I ($n \geqslant 4$)	II ($n \geqslant 5$)
Phencyclidine	240 ± 20	0 ± 3
4-OH-cyclo PCP	**	23 ± 3
4-OH-pip PIP	**	61 ± 7
(+)-SKF 10047	2·2 ± 0·3	21 ± 5
(−)-SKF 10047	4·1 ± 0·7	9 ± 1

* Approximate standard error calculated according to DeLean et al (1978).
** No inhibition was observed at 500 μM.

* Correspondence.

the enzyme at concentrations up to 500 μM (Table 1, I). Assuming competitive inhibition as previously reported (Kloog et al 1977), we calculated from our IC50 value that the K_i of PCP is 96 μM which is in close agreement with the K_i values of 80 and 78 μM previously reported (Maayani et al 1974; Kloog et al 1977). Both (+)- and (−)-SKF 10047 were potent inhibitors of acetylcholinesterase, having calculated K_i values of 0·9 and 1·6 μM, respectively. The lack of anticholinesterase activity by the behaviourally less potent PCP metabolites and the greater potency of (+)-SKF 10047 compared with (−)-SKF 10047 suggest that this activity may be relevant from a behavioural viewpoint. However, the observation that SKF 10047 is 60–100 times more potent as an anticholinesterase than PCP, but approximately one-half as potent as PCP in behavioural measures, argues against this hypothesis (Shannon 1981; Brady et al 1981).

In agreement with others we found that PCP was a modest inhibitor of [^{3}H]QNB binding to rat brain membranes (Table 1, II). We also found that 4-OH-pip PCP and 4-OH-cyclo-PCP were 1/6 and 1/2 as potent as PCP, respectively. Although Domino (1964) reported that the monhydroxylated metabolites of PCP had very little behavioural activity, Shannon (1981) reported that 4-OH-pip PCP was about 1/3 and 4-OH-cyclo 1/33, as potent as PCP in producing PCP like effects in the rat. It has also been reported that *cis*-4-OH-cyclo PCP is 1/50th as potent as PCP in producing motor incoordination in the mouse (Martin et al 1981). Since 4-OH-cyclo PCP was equipotent with (+)-SKF10047 and more potent than 4-OH-pip PCP as an anticholinergic we suggest that this activity is not a key element in the mechanism which underlies the behavioural activity of PCP.

Since the (−)-isomer of SKF 10047 is more potent than the (+)-isomer in inhibiting schedule dependent rates of responding in monkeys and rats (Brady et al 1981), and in inhibiting [^{3}H]QNB binding (Table II), it is possible that this activity is related to the inhibition of muscarinic binding sites in the brain. However, since the discriminative stimulus properties of PCP are stereospecific in favour of the (+)-isomer of SKF 10047, these data argue against the notion that the antimuscarinic activity of PCP mediates the production of the internal cues responsible for the ability of either rats or monkeys to discriminate between the drug (PCP) and non-drug (saline) state. The potential importance of the antimuscarinic properties of PCP is further diminished by the observation that behaviourally active doses of PCP result in peak brain concentrations of 0·8–1·3 μM (Woolverton et al 1980; Martin et al 1980). Since this inhibition is competitive and the concentrations of acetylcholine in the rat brain range from 15–60 μM (Racagni et al 1976), the calculated inhibition of the muscarinic binding site at these concentrations is minimal. Although it is possible that PCP may be concentrated at the cholinergic receptor, we have observed no significant difference in the concentration of PCP between brain areas (Johnson & Balster 1981) which differ greatly in their cholinergic receptor density (Yamamura & Snyder 1974).

Therefore it appears that both the apparent cholinergic and anticholinergic behavioural effects of PCP observed by our laboratory (Johnson & Wilkenfeld, 1981) and by others (Kanner et al 1975; Adams 1980) must be mediated by mechanisms other than blockade of muscarinic receptors or inhibition of acetylcholinesterase. For example, the potentiation of PCP by oxotremorine which we observed (Johnson & Wilkenfeld 1981) may be due to a PCP-induced increase in central muscarinic receptor density and/or affinity (Boggan et al 1981).

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Vehicle effects on ophthalmic bioavailability: the influence of different polymers on the activity of pilocarpine in rabbit and man

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Viscosity-increasing polymers are usually added to ophthalmic drug solutions, on the premise that an increased vehicle viscosity should correspond a slower elimination from the preocular area, and hence a greater transcorneal penetration of the drug into the anterior chamber. Many polymers have been screened in the attempt to determine the ideal polymer and viscosity, and to establish quantitative relationships between vehicle viscosity, retention time and ophthalmic bioavailability. The experiments are usually carried out by the different authors on rabbits or man, and this has generated some confusing and contradictory data, since the two species show important differences in ocular anatomy and physiology (e.g., different rates of blinking, tear secretion and turnover, drainage of instilled fluid, etc) that may produce different responses to vehicle viscosity. In spite of some diverging reports as the effect, in man and in rabbits, of the viscosity of the vehicle on bioavailability, there appears to exist an implicit agreement on the thesis that, within each species, vehicles prepared with different polymers should behave identically when compared on an equal viscosity basis (Patton & Robinson 1975). In the present study, three Newtonian equiviscous vehicles and one pseudoplastic vehicle, all prepared with different polymers and containing pilocarpine were tested on rabbits and man. The aims of the investigation were (a) to verify the equal viscosity-equal activity assumption, i.e., the alleged lack of influence on activity of the type of polymer; (b) to define species differences in the biological response to the same vehicles; and (c) to assess the relevance to activity of the type of flow of the vehicle (Newtonian *vs* pseudoplastic).

All preparations tested were made by adding the appropriate amount of polymer to an aqueous isotonic buffered (Sørensen 0·2 M phosphate buffer, pH 5·5) $2 \cdot 10^{-2}$ M solution of pilocarpine nitrate (AS) serving as reference. The following polymers, at the indicated w/v concentrations, were used: hydroxypropylcellulose, low molecular weight (HPCL, Klucel LF, Hercules Inc.), 5·0%; hydroxypropylcellulose, medium molecular weight (HPCM, Klucel MF, Hercules Inc.), 1·2%; polyvinyl alcohol, high (90·000) molecular weight (PVA, Polyviol W 48/20, Wacker Chemie), 5·0%; polyvinylpyrrolidone (PVP, Plasdone K 90, GAF), 6·0%. All solutions were sterilized by autoclaving at 2 bars for 20 min; their pH after sterilization was virtually unchanged, while the pilocarpine content (h.p.l.c.) showed an average 0·15% decrease. Viscosity determinations on the sterilized vehicles, made at 30°C on a Rheomat 30 rotary viscometer (Contraves AG) indicated for HPCL, PVA and PVP a Newtonian behaviour (rate of shear up to 700 s^{-1}) and a viscosity of 73 ± 2·5 cps.

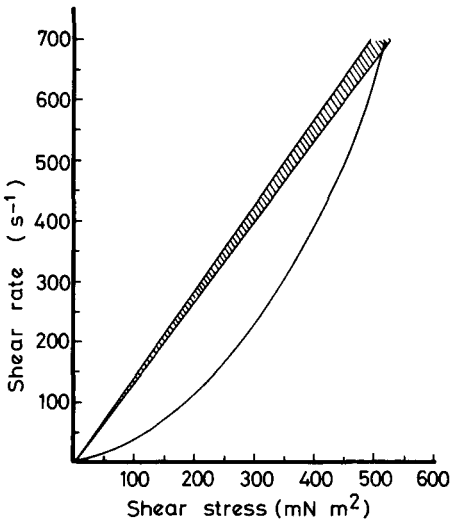

FIG. 1. Rheograms (30°) of the vehicles tested in the present study.

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