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PRELIMINARY NOTE

CORRELATION BETWEEN ANIMAL AND CLINICAL FINDINGS WITH A PSYCHOTOMIMETIC ANTICHOLINESTERASE

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Summary—RX 67668 (*cis*-2-phenyl-1-pyrrolidinocyclohexane hydrochloride, Reckitt and Colman), a novel, reversible anticholinesterase agent, was evaluated against eserine, pyridostigmine and LSD in rats in a Sidman discriminated avoidance test.

RX 67668, eserine and LSD decreased discriminated avoidance behaviour, increased late responding but did not significantly affect responding in the pre-signal period. Pyridostigmine, which penetrates the blood–brain barrier only with difficulty, had minimal effects on the three response categories.

On the basis of the behavioural profiles presented, it was predicted that RX 67668 might induce psychotomimetic effects in man. This conclusion was in agreement with findings from a concomitant clinical evaluation of the central effects of RX 67668.

The psychotomimetic effects of certain anticholinesterase drugs have previously been reported (DOWNING, 1964). Eserine can bring about striking sensory changes (KREMER, 1942) and the administration of di*iso*propylfluorophosphate in the treatment of myasthenia gravis is associated with nightmares, mental confusion and hallucinations (GROB, LILIENTHAL, HARVEY and JONES, 1947). Also, SHERWOOD (1958) has observed that neostigmine, again taken by myasthenic patients, occasionally produces psychotic episodes.

Since the Sidman avoidance technique is of use in the evaluation of psychotomimetic compounds (SMYTHIES, 1968; WRAY and COWAN, 1971), it was thought of interest to compare RX 67668 (*cis*-2-phenyl-1-pyrrolidinocyclohexane hydrochloride, Reckitt and Colman), a novel, reversible anticholinesterase intended for use in myasthenia gravis, with eserine and LSD. Pyridostigmine, which penetrates the blood–brain barrier to a lesser degree, was used as a control.

METHODS

Female hooded rats (200–220 g) were trained 5 days per week over 15 weeks on a Sidman discriminated avoidance schedule (SIDMAN, 1955). This procedure is described by referring to three time intervals: response—onset of warning signal (R–W), 20 sec; onset of warning signal—shock (W–S), 10 sec; shock—shock interval (S–S), 10 sec. In this free-operant situation all responses are equally effective in postponing shocks. Thus, responses during the R–W interval postpone the warning signal onset, and responses during the W–S period postpone the shock. A shock is delivered every 10 sec (S–S interval) until there is a lever press (designated as a "late response"). The recording and programming equipment used have been described previously (WRAY, 1972). Each session consisted of a 25 min "warming-up" period and a 120 min run. Each rat was injected at the end of the 25 min period.

Control sessions in which 0·9% saline was injected took place 24 hr before, and 48 hr after, each drug session.

The following compounds were administered subcutaneously in a random order to a group of 6 rats, with 14 days training occurring between treatments: RX 67668 hydrochloride (Reckitt and Colman) physostigmine sulphate (BDH); pyridostigmine bromide (Mestinon, Roche); D-tartrate of D-lysergic acid diethylamide (Lysergamide, Spofa; injected i.p.). All doses quoted are in terms of the respective salt.

RESULTS

The cumulative record (Fig. 1) illustrates the disruptive effects of RX 67668 (10 mg/kg), eserine (0·50 mg/kg) and LSD (0·50 mg/kg) on a well-established avoidance performance in rats.

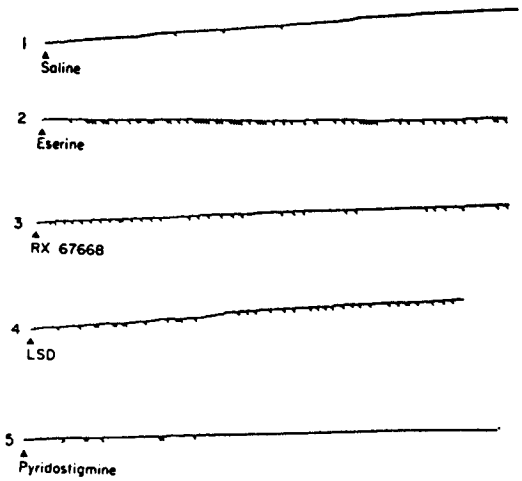

Fig. 1. Representative cumulative records of discriminated avoidance behaviour of various single rats. The abscissa represents the first 30-min time interval under the various drugs. The ordinate represents the cumulative number of responses after injection of 1. saline (2·5 ml/kg), 2. eserine (0·50 mg/kg), 3. RX 67668 (10 mg/kg), 4. LSD (0·50 mg/kg) and 5. pyridostigmine (0·50 mg/kg). The slope of each curve is proportional to the rate of bar pressing. Shocks are indicated by vertical "pips". Note the similarities in the traces of RX 67668 and LSD and of saline and pyridostigmine, respectively.

The data for each response category are presented in Figure 2 in the form of probabilities derived from inter-response times per opportunity statistic (IRTs/OPP). This statistic gives the probability of an avoidance response in the presence of the warning signal. Although RX 67668, eserine and LSD did not significantly ($P > 0·05$) affect responses in the R–W interval, rats injected with LSD tended to place more responses in this interval (Fig. 2). All three drugs increased the probability (IRTs/OPP) of a warning signal response (W–S interval) compared with their respective saline scores. This response decrement brought about an increase in late responding, all three drugs affecting avoidance but not escape responding.

An analysis of variance (3-way repeated measures) revealed that, over the 2 hr experimental session, there was no significant decrement in responses between the blocks (30 min intervals) for RX 67668 ($F < 1·0$), LSD ($F < 1·0$) or eserine ($F = 3·16$; d.f. = 2, 6; $P > 0·05$)

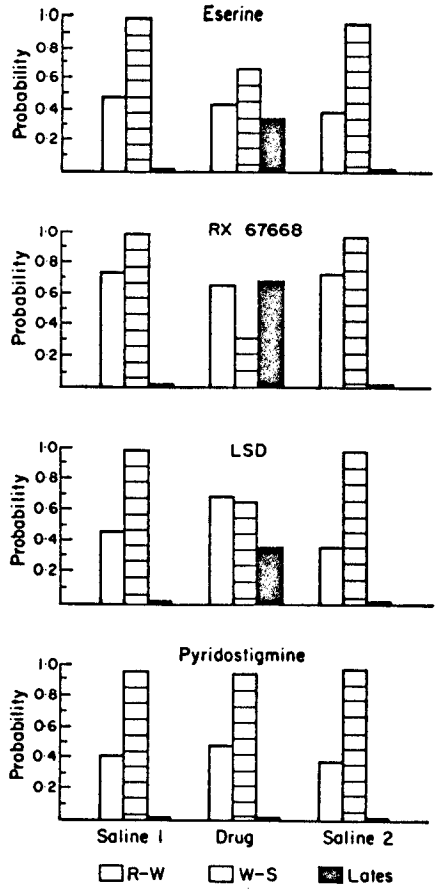

Fig. 2. The probability of an avoidance response from individual rats over 2 hr (ordinate) after administration of saline 24 hr before, and 48 hr after, either eserine (0·50 mg/kg), RX 67668 (10 mg/kg), LSD (0·50 mg/kg) or pyridostigmine (0·50 mg/kg) (abscissa). The response categories R–W, W–S and Lates are explained fully under "Methods". RX 67668, LSD and eserine selectively block conditioned avoidance responding whereas pyridostigmine has minimal effects on the frequency distribution of responses.

although for 60 min after eserine administration there was a significant depression of response rate ($F = 5{\cdot}88$; d.f. = 3, 6; $P < 0{\cdot}05$) (Table 1).

RX 67668 (1·0 mg/kg) and pyridostigmine (0·50 mg/kg) brought about minimal effects on the three response categories throughout the 2 hr session.

Table 1. Effect of eserine, RX 67668 and pyridostigmine on rate of responding

Time (min)	Eserine 0·50 mg/kg			RX 67668 10 mg/kg			Pyridostigmine 0·5 mg/kg		
	S_1	D	S_2	S_1	D	S_2	S_1	D	S_2
30	98·7	70·3	102·4	109·4	100·2	105·2	111·0	120·3	123·7
60	103·7	69·0	96·3	101·2	97·0	116·2	100·7	110·7	104·3
90	110·0	90·3	94·0	104·0	105·8	108·8	110·0	119·3	100·0
120	113·3	101·1	106·0	100·6	103·8	100·2	115·0	103·0	107·3

The mean number of responses per 30-min interval following the s.c. injection of eserine (n = 3), RX 67668 (n = 5) and pyridostigmine (n = 3) into rats trained on a Sidman discriminated schedule. Saline was injected 24 hr before (S_1) and 48 hr after (S_2) each drug (D).

DISCUSSION

RX 67668, LSD and eserine were similar in that each drug substantially decreased discriminated avoidance behaviour (W–S interval) and increased late responses. Although the three drugs did not significantly affect responding in the pre-signal period (R–W), responses were somewhat higher in rats receiving LSD.

RX 67668 and LSD had little effect on response rate within each 30 min interval whereas eserine induced a significant decrement for 60 min after injection. However, no drug produced a significant depression of rate over the 2 hr experimental session.

It was felt that the behavioural profiles of these compounds were sufficiently similar to indicate that RX 67668 might be psychotomimetic in man. This suggestion found confirmation in a concurrent and independent study of the central effects of RX 67668 in human volunteers. The administration of this compound (doses ranged from 0·40 to 1·0 mg/kg. i.v.) to 8 subjects induced disorientation, sensory disturbances, dysphoria, apprehension, emesis and hallucinations (GILLETT, HEDGES, METCALF, RICHENS and ROYDS, 1972). The high incidence of these side-effects has led to the withdrawal of RX 67668.

It is uncertain to what extent inhibition of cholinesterase *per se* is responsible for the behavioural disturbances observed with RX 67668. This property is common to psychotomimetics such as adrenochrome, bufotenine, LSD (HOFFER and OSMOND, 1967), levallorphan (LANE, MACFARLANE and MCCOUBREY, 1966) and, of course, di*iso*propylfluorophosphate. The latter compound, in fact, exacerbates the psychotic state in schizophrenic patients (ROWNTREE, NEVIN and WILSON, 1950). However, it is difficult to accept more than a tentative relationship since, as a class, anticholinesterases are not remarkable for their hallucinogenic properties and some (e.g. tetrahydroaminoacridine) are even capable of reversing the hallucinatory effects produced by the anticholinergic psychotomimetics (BELL, GERSHON, CARROLL and HOLAN, 1964).

It seems clear that the pharmacological basis for the behavioural action of a psychotomimetic anticholinesterase such as RX 67668 is as yet not established.

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