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Stereospecificity of behavioural effects of viloxazine in bulbectomized rats does not correlate with its 5-HT-releasing action in vitro

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The effects of the antidepressant drug viloxazine and its two optically active isomers on the passive avoidance learning deficit surgically induced in rats by bilateral bulbectomy have been determined. Fourteen consecutive daily injections of either the racemate or the *S*-isomer (2, 5 and 10 mg kg^{-1} i.p.) significantly improved the acquisition of avoidance behaviour. The *R*-isomer was devoid of such activity in this same dose range. The various isomeric species of viloxazine were also studied in vitro for their ability to induce a release of noradrenaline, dopamine and 5-hydroxytryptamine (5-HT) from rat brain slices. In agreement with previous reports, racemic viloxazine caused a concentration-dependent (10^{-5} – 10^{-3} M) release of 5-HT without affecting any significant change in the release of either catecholamine. However, in contrast to the results of the behavioural studies, this phenomenon did not exhibit stereospecificity, all three isomeric forms being equally active. Thus, there is no simple relationship between viloxazine's behavioural activity in bulbectomized rats and its 5-HT releasing properties observed in vitro. The significance of these findings with respect to previously reported effects of the drug indicative of facilitation of central 5-HT mediated processes is discussed.

Bilateral olfactory bulbectomy (OB) in the rat results in a behavioural and biochemical syndrome which is selectively reversed by repeated administration of clinically effective antidepressant drugs (Ueki et al 1972; Cairncross et al 1975, 1978). Behavioural changes reversed by antidepressants include the learning deficit in a step-down passive avoidance situation, hyperirritability and muricidal behaviour. Antidepressants also restore to normal levels the marked elevation in 11-hydroxycorticosteroids which also occur in the OB rat.

The neurochemical mechanisms underlying the development of the various features of the OB syndrome are poorly defined (Hirsch 1980). A reduction in 5-hydroxytryptaminergic neurotransmission has recently been invoked on the basis that several of the drugs which are effective in ameliorating the learning deficit, e.g. amitriptyline, mianserin, fenfluramine and fluoxetine, share a common property of stimulating 5-hydroxytryptamine (5-HT) receptors, albeit by indirect mechanisms (Broekkamp et al 1980). The antidepressant viloxazine has also been reported to reverse the OB syndrome (Cairncross et al 1979) a finding of particular interest in this context because there is some evidence that this drug may also facilitate central 5-HT mediated neurotransmission (Lippman & Pugsley 1976; Pawlowski et al 1979) possibly by a 5-HT releasing action (Martin et al 1978). Moreover, viloxazine exists as two optically active isomers which possess differing behavioural and biochemical properties. The optically active *S*-isomer (ICI 73,333) exhibits marked antireserpine and motor depressant activity (Howe et al 1976) and it is a highly selective inhibitor of noradrenaline uptake both in vitro and in vivo (Blackburn et al 1978). In contrast, the *R*-isomer (ICI 73,332) has little effect on behaviour and is inactive as an inhibitor of biogenic amine uptake. We were thus prompted to determine whether the ability of viloxazine to reverse the learning deficit in OB rats and to release 5-HT from brain slices in vitro also exhibits stereospecificity since such information could provide corroborative evidence for a role of 5-HT in the behavioural actions of viloxazine and by implication in the OB syndrome.

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METHODS

Behavioural studies in bulbectomized rats. Surgical preparation of OB rats was as described by van Riezen et al (1977). Both olfactory bulbs of male Wistar rats (200–220 g) of the Alderley Park SPF Strain II were stereotactically sectioned and aspirated under halothane anaesthesia. Sham operated (SO) controls were subjected to similar anaesthetic

and surgical procedures but with the omission of the sectioning and removal of the olfactory bulbs. Postoperatively OB and SO rats were housed in groups of five per cage for a period of 7 days. On the eighth day intraperitoneal administration of drug or 0·9% NaCl (saline) to both OB and SO rats was commenced and continued, once daily at 16.00 h, until the fifteenth post operative day. Throughout the morning of the fifteenth day all animals were subjected to a 'step-down' passive avoidance test (Cairncross et al 1978). The apparatus used consisted of a clear Perspex box with a 55 cm^2 stainless steel grid floor the bar centres of which were set at 1·5 cm intervals each connected to an electric stimulator capable of delivering 0·63 mA, 50 square wave pulses s^{-1}. A 19 cm^2 non-conductive Perspex platform was located in the centre of the box 4 cm above the steel grid. Immediately before testing, paws were moistened with saline and avoidance responses determined by placing the rat on the Perspex platform and measuring the step-down latency which was defined as the time interval between being placed upon the platform and the moment when all four paws were on the steel grid floor. This procedure was repeated with an intertrial interval of 30 s until the animal learned to remain on the platform for 2 min or for a maximum of 10 trials. The number of trials required for each rat to attain this criterion was determined. Statistical significance between groups was determined by means of the Mann Whitney U-test (Goldstein 1964).

Amine release from rat brain slices. Slices of specific brain areas of the rat were prepared by chopping in two directions at 100 μm intervals using a McIlwain Tissue Chopper. The slices were incubated at 37 °C for 15 min in 5 ml Krebs buffer (gassed with 95%/5% O_2/CO_2 to pH 7·4) in the presence of 0·1 μM [^{3}H] neurotransmitter (5-HT, noradrenaline, dopamine) and 1 μM pargyline; 0·1 mM ascorbic acid was also present in the noradrenaline and dopamine studies. The slices were washed three times with Krebs buffer and allowed to equilibrate to a low basal rate of efflux of [^{3}H] neurotransmitter by standing in Krebs buffer for 30 min. After a further wash, aliquots (100 μl) of the Krebs containing the slices were incubated in a final volume of 1 ml in Krebs buffer at 37 °C in the presence or absence of the drug for 10 min followed by centrifugation in an Eppendorf Centrifuge for 1 min. 500 μl amounts of the supernatnt and 500 μl of a solution of the tissue in 1 ml PCS scintillation fluid were added to 4 ml of PCS and counted in a liquid scintillation spectrophotometer. The extent of the release induced by the drug was estimated by measuring the % of the total radioactivity released into the supernatant over the 10 min incubation. Basal rates of release in the absence of drugs were in the range of 10–20% in the 10 min studied. [^{3}H] 5-HT and [^{3}H] noradrenaline release were studied using slices of the frontal cortex; [^{3}H] dopamine release was studied using slices of the striatum. Radiochemicals used were [^{3}H] 5-HT (28·9 Ci $mmol^{-1}$, New England Nuclear); [^{3}H] adrenaline (13 Ci $mmol^{-1}$, Amersham); [^{3}H] dopamine (43 Ci $mmol^{-1}$, Amersham).

RESULTS

The performance of OB rats for learning the passive avoidance criterion was significantly impaired in comparison with SO controls (7·5 ± 0·1 c.f. 3·6 ± 0·1 trials to criterion (Table 1) and this learning deficit was significantly attenuated by prior administration of viloxazine (5 and 10 mg kg^{-1} i.p. on days 8–14 inclusive). Viloxazine had no significant effect on the ability of SO rats to acquire the avoidance response.

A comparison of the effects of racemic viloxazine with those obtained with its two optically active isomers in OB rats is shown in Fig. 1. Drug effects are expressed as a percentage of the appropriate

Table 1. Effects of olfactory bulbectomy and viloxazine on passive avoidance learning in the rat. The figures indicate the number of trials required (mean ± s.e.m.) for each group of sham operated (SO) and olfactory bulbectomized (OB) rats to acquire the passive avoidance response as defined in Methods. * Statistical significance with respect to the appropriate controls was assessed using the Mann Whitney U-test.

Surgical treatment	Drug (mg kg^{-1} i.p.)	No. of animals	No. of trials to criterion mean ± s.e.m.	P^*
SO	Saline	5	3·6 ± 0·1	—
SO	Viloxazine (5)	5	3·6 ± 0·7	NS
SO	Viloxazine (10)	5	3·4 ± 0·3	NS
OB	Saline	26	7·5 ± 0·1	—
OB	Viloxazine (5)	10	3·9 ± 0·1	<0·01
OB	Viloxazine (10)	7	2·6 ± 0·1	<0·01

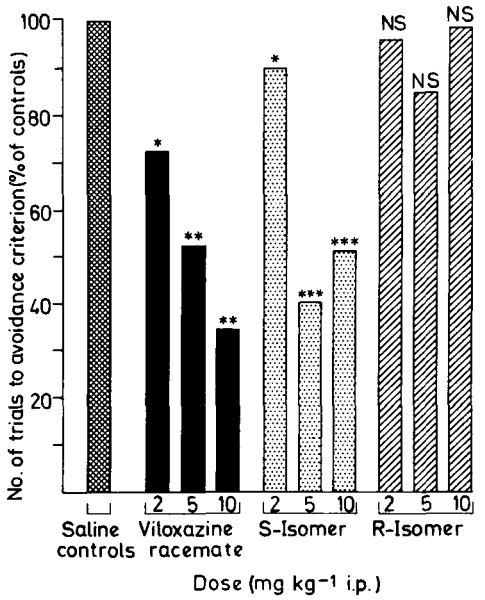

FIG. 1. Effect of viloxazine and its optical isomers on passive avoidance in bulbectomized rats. The columns represent the mean number of trials (expressed as a percentage of saline treated controls) required for drug-treated bulbectomized rats to acquire the passive avoidance response as defined in Methods.

saline control response. The response to the racemic mixture of viloxazine exhibits a dose-dependent relationship at 2·5 and 10 mg kg^{-1}. Furthermore, results obtained with the two optical isomers over this same dose range reveal a distinct separation of activity. The *S*-isomer improves the acquisition of avoidance behaviour at all dose levels, whereas the *R*-isomer is devoid of behavioural activity in the passive avoidance test. The effects of racemic viloxazine and the *S*-isomer were not significantly different (Fig. 1). There were no behavioural effects detectable in SO rats.

The results of the biogenic amine release experiments in vitro (Table 2) indicate that relatively high concentrations of viloxazine induce a concentration-dependent release of 5-HT from the frontal cortex. This effect appears to be selective for 5-HT since at 10^{-4} M noradrenaline and dopamine release from the frontal cortex and striatum respectively are not affected. In contrast to the findings in the passive avoidance experiments in vivo, the 5-HT releasing action of viloxazine appears to be a common property of both *S* and *R*-isomers with no apparent difference between either optically active species or the racemic mixture.

Table 2. Effect of viloxazine and its optical isomers on the release of [^{3}H] amines from rat brain slices. * Results are the mean ± s.e.m. of three individual determinations.

	% released above basal in a 10 min incubation period		
Drug (M)	[^{3}H] 5-HT (Frontal cortex)	[^{3}H] NA (Frontal cortex)	[^{3}H] DA [^{3}H] DA (Striatum)
Viloxazine			
10^{-3}	60·3 ± 3·2 ***	ND	ND
10^{-4}	7·9 ± 0·7 ***	0	0
10^{-5}	2·3 ± 0·8 *	0	0
R-isomer			
10^{-3}	61·0 ± 1·2 ***	ND	ND
10^{-4}	9·5 ± 0·9 ***	0	0
10^{-5}	1·1 ± 0·7 NS	0	0
S-isomer			
10^{-3}	55·1 ± 0·2 ***	ND	ND
10^{-4}	8·6 ± 0·3 ***	0	0
10^{-5}	0·3 ± 0·4 NS	0	0

ND = Not determined.

Statistical significance with respect to basal release rates in the absence of drug was assessed using Student's *t*-test (* $P < 0.05$, *** $P < 0.001$, NS not significant).

DISCUSSION

The results of this study confirm several previous reports that OB impairs the acquisition of a passive avoidance response (Cairncross et al 1975, 1979; van Riezen et al 1977) and that the development of this behavioural deficit is prevented by the chronic daily administration of, amongst various antidepressants, viloxazine (Cairncross et al 1979, 1980). In addition, the present data indicate that when administered as the racemic mixture this property is attributable exclusively to an action of the optically active *S*-isomer; the *R*-isomer having no observable effects on the acquisition of the passive avoidance response by OB rats over the dose range investigated.

The brain slice amine release studies are in agreement with a previous report for a selective release of 5-HT by racemic viloxazine at 10^{-5} M (Martin et al 1978). Since viloxazine has previously been shown to be a selective inhibitor of noradrenaline uptake (Blackburn et al 1978) and in the present studies is devoid of noradrenaline-releasing activity at 10^{-4} M, it is unlikely that the observed effects on 5-HT release are a consequence of uptake inhibition. This 5-HT releasing action is distinguished from the behavioural effects described here in that it does not display stereospecificity. It therefore appears from the present data with the various isomeric forms of viloxazine, that there is no simple relationship between this drug's behavioural activity in the OB

rat model and its 5-HT releasing properties observed in vitro. Moreover, it is uncertain whether administration of viloxazine at the dose showing behavioural activity would achieve the relatively high brain concentrations which appear to be necessary to cause a significant release of 5-HT. However, the possibility of accumulation during chronic administration cannot be excluded. Nevertheless, an action of viloxazine via 5-HT mediated mechanisms has been suggested previously on the basis of enhanced behavioural responses to 5-HTP (Lippman & Pugsley 1976), facilitation of the hind-limb flexor reflex of the spinal rat (Pawlowski et al 1979) and potentiation of 5-HT evoked responses of rat cortical neurons (Jones & Roberts 1977). It is not known, however, whether all of these actions exhibit stereospecificity. An alternative interpretation of the present data, and one which accommodates the behavioural stereospecificity observed, is that the effects seen in OB rats are partly dependent upon, or are modulated by, enhanced noradrenergic neurotransmission resulting from the known stereospecific inhibition of noradrenaline uptake (Blackburn et al 1978). It is also possible that the behavioural stereospecificity is a reflection of differences in the pharmacokinetic or metabolic profiles of the optical isomers and that amine release studies in vitro are not an accurate representation of drug induced changes in 5-HT release in vivo.

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