Effects on the pinna reflex of drugs acting at α-adrenoceptors

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Several classes of centrally-acting drugs, including muscle relaxants, hypnotics and neuroleptics, inhibit the pinna-reflex in mice (Witkin et al 1959; Corne et al 1963). We have investigated the effects of a range of drugs acting at α-adrenoceptors and have found α_2-adrenoceptor agonists to be potent inhibitors of the reflex. This inhibition was abolished by α_2-adrenoceptor antagonists but not by prazosin, an antagonist selective for α_1-adrenoceptors.

Male TO mice (20–30 g) were kept in a quiet experimental room for at least 6 days before the experiments which took place between 1200 and 1630 h. The pinna reflex was tested at 20, 40, 60, 90, 120 and 180 min after injection of α-adrenoceptor agonists. Testing was carried out in the experimental cage (polypropylene 24 × 18 × 10 cm) by stimulating each auditory meatus with a fine wire (Witkin et al 1959) without handling the mouse. Animals were considered to show a pinna reflex if they responded to stimulation of either ear by shaking the head. All control animals showed a pinna reflex. ED50 values (Litchfield & Wilcoxon 1949) for inhibition of the pinna reflex were determined at the time of peak effect of each agonist. For examination of the effects of antagonists, a dose of each agonist was chosen which produced just submaximal (80–90%) inhibition: the ID50 of the antagonist was expressed as the dose producing 50% restoration of the pinna reflex. The antagonists were administered 15 min after the agonist except where the latter was guanfacin or centrally injected oxymetazoline when the antagonist was given after 60 min. All drugs were injected subcutaneously (s.c.) in 0·9% NaCl (saline); oxymetazoline and methoxamine were injected into the cerebral ventricles (i.c.v.) by the method of Brittain & Handley (1967). Doses are expressed in terms of the salt: clonidine HCl (Boehringer); guanabenz acetate (Wyeth); guanfacin HCl (Sandoz); oxymetazoline HCl (Merck, Ltd); methoxamine HCl (Burroughs Wellcome); piperoxane HCl (May & Baker); yohimbine HCl (Sigma) and prazosin HCl (Pfizer).

The effects of the five α-adrenoceptor agonists are shown in Table 1; when given s.c., clonidine, guanfacin and guanabenz inhibited the pinna reflex in a dose-dependent manner, while methoxamine and oxymetazoline were ineffective. The latter two drugs were therefore injected i.c.v. Methoxamine then partially inhibited the pinna reflex producing 50% inhibition at both 10 and 20 μg i.c.v.: at these doses mice exhibited behaviour compatible with extreme fearfulness, remaining immobile but highly alert with ears pressed back. Oxymetazoline i.c.v. (2·5–5·0 μg) had no effect until 60 min after injection when inhibition appeared progressing to 100% by 180 min; in addition, a marked behavioural syndrome developed with the same time course as the pinna reflex inhibition. This syndrome consisted of stereotyped head-searching, sniffing, compulsive locomotion and severe hind-limb splay. Backward locomotion occurred in a few animals.

The effects of the three α-adrenoceptor antagonists on inhibition of the pinna reflex produced by the five agonists are shown in Table 2. When given alone, yohimbine (0·05–2·5 mg kg^{-1} s.c.), piperoxane (0·1–10·0 mg kg^{-1} s.c.) and prazosin (0·5–5·0 mg kg^{-1} s.c.) were without effect on the incidence of the pinna reflex, although it appeared to be more readily elicited and of greater amplitude after yohimbine and piperoxane than in saline-injected controls. Yohimbine and piperoxane produced a dose-dependent reversal of the inhibition of the pinna reflex due to submaximal doses of clonidine, guanfacin, guanabenz s.c. and oxymetazoline i.c.v.; the partial inhibition produced by methoxamine 20 μg i.c.v. was also prevented. Except for a slight antagonism of clonidine, prazosin was without effect on inhibition of the pinna reflex by the agonists. At 2·5 mg kg^{-1} prazosin partially (22%) reversed clonidine-inhibition but 5·0 mg kg^{-1} prazosin did not produce any greater effect.

Inhibition of the pinna reflex is a property of many classes of centrally-acting drugs but, with the exception of neuroleptics, certain narcotics and, to a lesser extent, mephenesin and ethanol, it only occurs at doses

Table 1. The effects of α-adrenoceptor agonists on pinna reflex.

Drug	ED50 for inhibition of pinna reflex with 95% confidence limits measured at time of peak activity	n*	Time after inj. (min)
Clonidine	0·275 (0·17–0·43) mg kg^{-1} s.c.	10	40
Guanfacin	1·12 (0·67–1·88) mg kg^{-1} s.c.	10	180
Guanabenz	1·12 (0·65–1·94) mg kg^{-1} s.c.	10	60
Oxymetazoline	> 10 mg kg^{-1} s.c. **	5	up to 180
	2·9 (1·84–4·58) μg i.c.v.	at least 7	150
Methoxamine	> 25 mg kg^{-1} s.c. **	5	up to 180
	Approx. 15 μg i.c.v. †	at least 6	20

* number of animals per dose
** i.e. no inhibition up to dose stated
† a maximum of 50% inhibition was obtained with both 10 and 20 μg i.c.v.

* Correspondence.

Table 2. The effects of α-adrenoceptor antagonists on the inhibition of the pinna reflex produced by α-adrenoceptor agonists.

Agonist used	ID50 of antagonist with 95% confidence limits in mg kg^{-1} s.c. measured at time of peak activity of agonist (see Table 1)		
	Yohimbine	Piperoxane	Prazosin
Clonidine 1 mg kg^{-1} s.c.	0·85 (0·5–1·45)	0·2 (0·11–0·36)	14·2**
Guanfacin 2·5 mg kg^{-1} s.c.	0·14 (0·08–0·24)	1·3 (0·62–2·73)	—*
Guanabenz 2·5 mg kg^{-1} s.c.	0·58 (0·34–1·10)	0·23 (0·15–0·36)	—*
Oxymetazoline 5 μg i.c.v.	1·45 (0·99–2·13)	5·4 (4·06–7·18)	—*
Methoxamine 20 μg i.c.v.	0·9 (0·62–1·31) †	Approx. 2·0 †	—*

* no antagonism up to 5 mg kg^{-1} prazosin
** partial antagonism only—maximal reversal was 22% with 1, 2·5 and 5 mg kg^{-1}. ID50 estimated.
† ID50 values are not comparable with effects on other agonists because maximum inhibition of pinna reflex by methoxamine was 50%.

sufficient to cause gross behavioural impairment (Witkin et al 1959; Corne et al 1963). This was not the case with the α-adrenoceptor agonists. At the ED50 for inhibition of the pinna reflex, clonidine, guanabenz and guanfacin caused at the most only mild sedation (assessed as described by Drew et al 1979). Methoxamine and oxymetazoline showed no sedative effect following either s.c. or i.c.v. administration.

The potency of neuroleptics in inhibiting the pinna reflex (Witkin et al 1959; Corne et al 1963) suggests the possible involvement of dopaminergic mechanisms. The present results show that α-adrenoceptors may also be important. Peripherally, α_1 and α_2-adrenoceptors have been distinguished by their differential sensitivity to agonists and antagonists. Thus, α_1-adrenoceptors are considerably more sensitive to the agonists methoxamine and oxymetazoline (Drew 1976) and the antagonist prazosin (Cavero et al 1977; Doxey et al 1977), while α_2-adrenoceptors are relatively more sensitive to clonidine, guanfacin and guanabenz (Drew 1976; Doxey 1979; Docherty et al 1979) and the antagonists yohimbine and piperoxane (Starke et al 1975; Doxey et al 1977). The effects of these drugs on the pinna reflex therefore suggest that they are acting upon receptors resembling the peripheral α_2-adrenoceptors. Clonidine, guanfacin and guanabenz were potent inhibitors of the reflex, while methoxamine was only partially effective even after central injection. The effect of oxymetazoline i.c.v. may well have been due to a metabolite in view of the delayed onset and unusual behavioural effects not seen with the other agonists. The two antagonists with selectivity for α_2-adrenoceptors, yohimbine and piperoxane, were potent in antagonizing the inhibitory effects of all the agonists while prazosin was ineffective even at doses well in excess of those shown to cause central effects in the mouse (Brown & Handley 1979).

If these preliminary results are confirmed, it may be possible to develop the pinna reflex test as a model system for evaluation of the central effects of α_2-adrenoceptor agonists and antagonists, with the advantage that it would be both rapid and simple to perform. Such a test would also be easy to incorporate into existing initial screening programs, as an aid to detection of agents with possible clonidine-like activity.

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