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The pinna reflex and its inhibition by clonidine: relationship to sedation and quantitation of central α_2-antagonist potency

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The relationship between sedation and pinna reflex inhibition has been measured for a range of centrally acting drugs. Ability to abolish the pinna reflex was not related to sedative activity as assessed by a behavioural method. Thus, at equisedative doses, diazepam, haloperidol, mianserin, prazosin and indoramin failed to abolish the pinna reflex while phenobarbitone and chlorpromazine caused partial- and clonidine complete-inhibition. At the ED50 for pinna reflex inhibition, guanabenz and guanfacine were significantly less sedative than clonidine. Mepyramine, yohimbine, RS-21361, idazoxan and phenylephrine produced little or no sedation and did not inhibit the reflex. When these agents (except for guanabenz and guanfacine) were tested for their ability to prevent clonidine-induced pinna reflex inhibition, all except the drugs with α_2-adrenoceptor antagonist activity were inactive. The potency order of the active agents was idazoxan > yohimbine > RS-21361 = mianserin. Antagonism of clonidine-induced pinna reflex inhibition may therefore prove to be a useful quantitative model for assessing the central potency of α_2-adrenoceptor antagonists.

α-Adrenoceptor agonists inhibit the pinna reflex with a potency order indicative of an effect at α_2-adrenoceptors (Brown & Handley 1980). However, certain other drugs also abolish the pinna reflex, notably among the neuroleptics, analgesics and sedative-hypnotics (Witkin et al 1959; Corne et al 1963). All these agents have sedative properties. It was therefore useful to establish whether this sedation could itself be responsible for inhibiting the pinna reflex. The relationship between sedation and pinna reflex inhibition has therefore been determined for a variety of centrally-acting drugs.

Since α_2-adrenoceptor antagonists reverse clonidine-induced inhibition of the pinna reflex in a dose-dependent manner, while prazosin was inactive (Brown & Handley 1980), this model could also prove useful to assess the central potency of agents known to possess peripheral α_2-adrenoceptor antagonist activity. This method has therefore also been used to assess the potency of a number of drugs, including α-adrenoceptor ligands of varying selectivity for α_1- and α_2-adrenoceptors.

Methods

Male albino TO mice (20–30 g) were used. Sedation was assessed in groups of 6 mice by the method of Drew et al (1979) in which ptosis, lowered body posture, slow gait, depressed touch-escape, depressed visual investigation of moving object, passivity to handling and impairment of righting reflex were scored by an observer who was 'blind' to the drug treatments given. Scores were on a 0–8 scale giving a total possible score of 56 for each animal. The pinna reflex was tested during this observation period as described by Brown & Handley (1980). All drugs were given s.c. except for phenobarbitone which was injected i.p. Mice were observed from 30–40 min after injection except those given guanabenz and guanfacin and those were assessed from 60–80 min, when the pinna reflex inhibition was maximal.

Reversal of clonidine-induced inhibition of the pinna reflex was measured in groups of 10 mice as described by Brown & Handley (1980), 40 min after clonidine 1·0 mg kg^{-1}. Drugs were administered s.c. 15 min before clonidine and the ID50, the dose producing 50% restoration of the pinna reflex, was calculated from regression analysis of the log-probit dose response curves.

Drugs. Drugs used were: clonidine HCl (Boehringer-Ingelheim), chlorpromazine HCl (May & Baker), diazepam (Roche), guanabenz acetate (Wyeth), guanfacin HCl (Sandoz), haloperidol (Janssen), idazoxan (Reckitt & Coleman), indoramin HCl (Wyeth), mepyramine maleate (May & Baker), mianserin HCl (Organon), phenobarbitone Na (Courtin & Warner), phenylephrine HCl (Sigma) Prazosin HCl (Pfizer) RS-21361 (2-(1-ethyl-2-imidazolylmethyl)-1,4-benzodioxan, Syntex). Drugs were dissolved or suspended in 0·9% NaCl except for haloperidol which was dissolved in a minimal volume of lactic acid and made up to volume in distilled water.

Results

Sedative effects in relation to pinna-reflex abolition. Fig. 1 shows the degree of sedation induced by the drugs tested, together with the number of mice in each group in which the pinna reflex was abolished. Saline solution, which was also included in the 'blind' behavioural analysis, gave a low positive score. Clonidine (0·25 mg kg^{-1}), guanabenz (1·12 mg kg^{-1}) and guanfacine (1·12 mg kg^{-1}) were tested at the previously reported ED50 for pinna reflex inhibition. At these doses, clonidine was significantly more sedative than were guanabenz or guanfacine ($P < 0·05$ in each case, Mann-Whitney 'U'-test). The most profound sedative effects were observed after diazepam, phenobarbitone and chlorpromazine; the highest doses of these three

agents produced similar degrees of sedation. However diazepam did not abolish the pinna reflex in any animals while phenobarbitone and chlorpromazine produced varying degrees of partial inhibition.

In doses equisedative with 1·0 mg kg^{-1} clonidine (Fig. 1) the drugs diazepam, haloperidol, mianserin, prazosin and indoramin were inactive in inhibiting the pinna reflex, while phenobarbitone and chlorpromazine caused partial inhibition. Clonidine, on the other hand, abolished the pinna reflex in all animals at this dose. Although chlorpromazine 5·0 mg kg^{-1} abolished the pinna reflex in 50% of mice, haloperidol at an equisedative dose was without effect. Mepyramine at the doses tested neither abolished the reflex nor caused any marked degree of sedation. Phenylephrine was similarly inactive.

Antagonism of clonidine-induced pinna reflex inhibition. ED50 values for antagonism of the clonidine-induced inhibition of the pinna reflex are shown in Table 1. Mepyramine, diazepam, phenobarbitone, chlorpromazine, haloperidol, prazosin and indoramin were inactive up to the doses shown. Although a response was shown by some of the mice pretreated with phenylephrine, it was not a typical pinna reflex, consisting rather of a withdrawal movement of head and shoulders followed by an orienting response. This response was not dose related, being maximal (20%) at 2·5 and absent at 4·0 mg kg^{-1}. The potency order of the remaining drugs was idazoxan > yohimbine > RS-21361 = mianserin.

Table 1. ID50 values for antagonism of clonidine (1·0 mg kg^{-1}) induced pinna reflex inhibition.

Drug	ID50 (mg kg^{-1})	95% confidence-limits	Relative potency (yohimbine = 1)
Yohimbine	0·79	0·49–1·26	1·0
RS-21361	10·8	7·1–16·5	0·07
Idazoxan	0·589	0·393–0·884	1·34
Mianserin	10·3	7·4–14·3	0·08
Indoramin	>50·0*	—	—
Prazosin	>2·5*	—	—
Phenylephrine	>4·0*	—	—
Mepyramine	>40·0*	—	—
Diazepam	>5·0*	—	—
Phenobarbitone	>90·0*	—	—
Chlorpromazine	>7·5*	—	—
Haloperidol	>1·0*	—	—

* Inactive up to dose shown.

Discussion

Inhibition of the pinna reflex was not related to the degree of sedation. Witkin et al used a range of sedative drugs and found that, although producing severe sedation, chlorpromazine, trifluperazine, mephenesin and ethanol were able to induce inhibition of the pinna reflex with intact righting reflexes. The present work

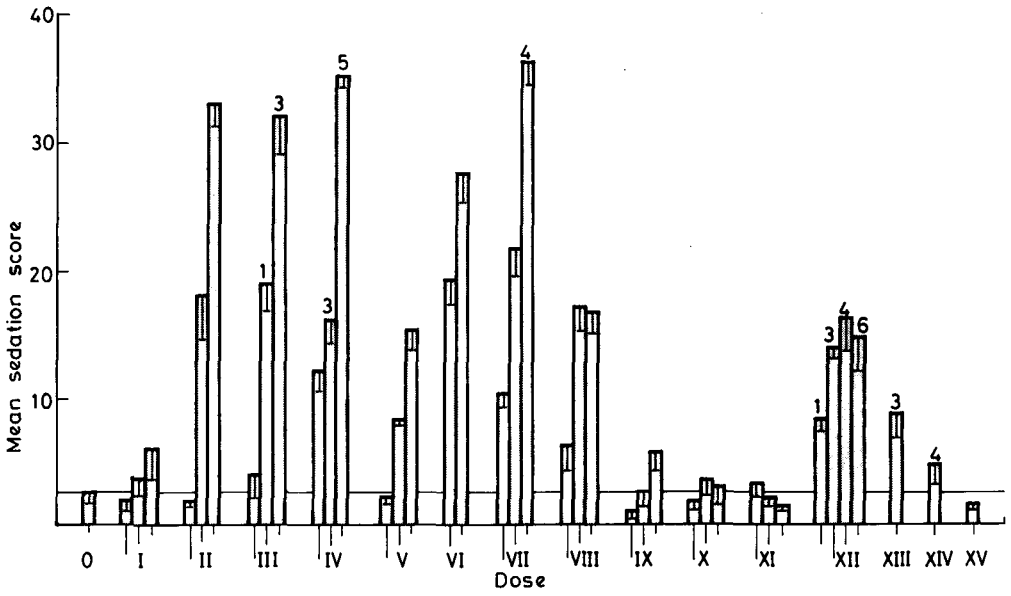

FIG. 1. Sedative effects in relation to the incidence of pinna reflex abolition (n = 6), vertical lines represent standard errors; numbers represent the incidence of pinna reflex inhibition in each group. No figure = zero inhibition. Drugs and doses in mg kg^{-1}: ○ Saline I, Mepyramine 10, 20, 40. II, Diazepam 1·25, 2·5, 5·0. III, Phenobarbitone 30, 60, 90. IV, Chlorpromazine 2·5, 5·0, 7·5. V, Haloperidol 0·125, 0·5, 1·0. VI, Prazosin 1·0, 2·5. VII, Indoramin 12·5, 25, 50. VIII, Mianserin 10, 20, 40. IX, Yohimbine 1, 5, 10. X, RS-21361 5, 10, 20. XI, Idazoxan 0·625, 1·25, 2·5. XII, Clonidine 0·25, 0·5, 1·0. XIII, Guanabenz 1·12. XIV. Guanfacine 1·12. XV, Phenylephrine 4.

amplifies these findings. Thus, in the first series of experiments, clonidine in a dose (1 mg kg^{-1}) close to the ED90 for pinna reflex inhibition (Brown & Handley 1980), abolished the reflex in all mice, while producing only 25% of the maximum sedation score; however, in equisedative doses, diazepam, haloperidol, mianserin, indoramin and prazosin were inactive in inhibiting the reflex. The α_2-adrenoceptor agonists clonidine, guanabenz and guanfacine were evaluated at the previously determined ED50 for inhibition of the pinna reflex (Brown & Handley 1980) and at these doses, showed considerable variation in the degree of sedation produced.

The remaining two drugs, phenobarbitone and chlorpromazine, did produce some inhibition of the reflex at doses equisedative with 1 mg kg^{-1} clonidine although they were much less potent in inhibiting the reflex. Witkin et al (1959) found, among a group of barbiturates, that there was severe neurological impairment including loss of righting reflex at the pinna reflex ED50. It seems likely therefore that, in this class of agent, pinna reflex loss cannot be discriminated from neurological impairment. The potency of chlorpromazine in inhibiting the pinna reflex is well documented (Witkin et al 1959; Corne et al 1963). In contrast, another neuroleptic, haloperidol, was inactive. Corne et al (1963) investigated several neuroleptic agents and found a potency order for inhibition of the reflex of chlorpromazine > trifluoperazine > perphenazine > thioridazine. This does not correspond to their potency in inhibiting dopamine receptors (Creese et al 1978). It is also not possible to account for the relatively high potency of chlorpromazine compared with other neuroleptics in terms of its antihistaminic or anti-5-hydroxytryptaminergic actions. A wide range of such agents has been shown to be inactive in antagonizing the pinna reflex (Corne et al 1963) and additionally we have shown mepyramine to have no effect. Anti-acetylcholine activity also appears to be an unlikely candidate in view of the low potency of thioridazine (Corne et al 1963). The cause of the high potency of chlopromazine compared with other neuroleptics therefore remains to be determined.

Among the α-adrenoceptor agonists, the inactivity of phenylephrine confirms the specificity of the pinna reflex inhibition for α_2-adrenoceptors. The doses of phenylephrine used are capable of affecting the central nervous system since they have marked effects on the head-twitch induced by intraventricular 5-hydroxytryptamine (Handley & Brown 1982). Phenylephrine is highly specific for α_1-adrenoceptors (Drew 1976).

These results show that, among the drugs tested, ability to inhibit the pinna reflex in the absence of severe sedation was strongly, though not exclusively, indicative of central α_2-agonist activity.

Among the drugs tested, only mianserin, idazoxan and RS-21361 antagonized clonidine-induced inhibition of the pinna reflex. Idazoxan (Doxey et al 1983) and RS-21361 (Michel et al 1981) are highly selective for α_2-adrenoceptors, although idazoxan has recently been reported to possess α_1-adrenoceptor agonist activity (Paciorek & Shepperson 1983). The α-adrenoceptor antagonist activity of mianserin is not selective between α_1- and α_2-adrenoceptors (Doxey et al 1978). The inactivity of indoramin (Rhodes & Waterfall, 1978) confirms previous findings for prazosin (Brown & Handley 1980) indicating the lack of involvement of α_1-adrenoceptors. The sedative effects of these agents suggest that they are entering the cns. Moreover, in this dose-range, prazosin profoundly inhibits the head-twitch response to intraventricular 5-hydroxytryptamine (Handley & Brown 1982).

Various methods have been used to estimate the central potency of α_2-adrenoceptor antagonists. Most have depended on the reversal of aspects of clonidine-induced sedation (e.g. Delini-Stula et al 1979; Drew et al 1979; Dettmar et al 1981), although there appears to be some controversy whether this sedation is exerted through an α_2-adrenoceptor (Clough & Hatton 1981). Reversal of clonidine-induced sedation is not suitable for drugs which have appreciable antagonist effects at α_1-adrenoceptors since blockade of these receptors potentiates clonidine-induced sedation (Delini-Stula et al 1979). This is clearly demonstrated in the case of mainserin, which is not selective between α_1- and α_2-adrenoceptors (Doxey et al 1978); mianserin failed to antagonize clonidine-induced suppression of exploratory activity in doses up to 100 mg kg^{-1}. In the present experiments, mianserin demonstrated clearcut dose-related antagonism of the effects of clonidine with an ED50 in the region of 10 mg kg^{-1}.

Since it appears that α_1-adrenoceptors are not involved in modulating the pinna reflex, estimates of central α_2-adrenoceptor antagonist potency obtained by this method should be independent of any inhibitory effects at α_1-adrenoceptors. It also means that the effects of clonidine on α_1-adrenoceptors should not interfere with the test. This may therefore be a useful means of assessing the central potency of drugs with proven activity at peripheral α_2-adrenoceptors.

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Is the hypothermic effect of α-methyldopa mediated by opioid peptides?

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At an ambient temperature of 21 °C α-methyldopa (25–200 mg kg^{-1}) induces a dose-dependent decrease in body temperature in rats. A relationship between adrenergic and opioid neuronal systems has been reported. Therefore, in this study the hypothesis that α-methyldopa produces hypothermia through release of endogenous opioid peptides has been investigated using the opiate antagonist naltrexone. The hypothermic effect of α-methyldopa is potentiated by naltrexone pointing to antagonism of an hyperthermic acting opioid system. However, at an ambient temperature of 6 °C, pretreatment with naltrexone did not significantly alter the hypothermic effect of α-methyldopa. Although the hypothesis proved not to be correct, it is concluded that depending on ambient temperature opioid peptides are involved in the determination of the ultimate effect of α-methyldopa on body temperature in rats.

Several neuronal systems are involved in the maintenance of constant body temperature in mammals. Both classical neurotransmitters such as noradrenaline, 5-hydroxytryptamine, dopamine and histamine and neuropeptides such as endorphins, substance P, cholecystokinin, bombesin and somatostatin have been studied from this aspect. The centrally acting adrenergic agonists α-methyldopa (Nijkamp et al 1975) and clonidine (Laverty & Taylor 1969; Zacny 1982) have been shown to lower body temperature in rats. The opioid peptide β-endorphin, after intracerebroventricular administration, also causes changes in body temperature in rats, high doses resulting in hypothermia and low doses in hyperthermia (Tseng et al 1979). Evidence is accumulating for a relationship between adrenergic and opioid systems. The fall in blood pressure observed after administration of α-methyldopa and its active metabolite α-methylnoradrenaline is prevented by pretreatment with the opiate antagonist naloxone (Petty & de Jong 1982) and the α-agonist clonidine causes analgesia in rats (Paalzow & Paalzow 1976; Fielding et al 1978) and alleviates opiate withdrawal symptoms in morphine-treated animals (Fielding et al 1978). Furthermore, adrenergic agonists such as α-methylnoradrenaline cause release of opioid peptides from the pituitary of the rat both in-vivo and in-vitro (Pettibone & Mueller 1982). For these reasons we investigated a possible interaction between α-adrenergic and endorphin systems involved in the regulation of body temperature in the rat by using the centrally acting α-agonist α-methyldopa and the opiate antagonist naltrexone.

Methods

Male Wistar rats (Wu-Cpb, Central Breeding Laboratories TNO, Zeist, The Netherlands), 225–275 g, were maintained at an ambient temperature of 22 °C. During the experiments the rats were individually housed in a quiet room at an ambient temperature of 21·0 ± 0·5 °C or 6·0 ± 0·5 °C. After an adaptive period of 30 min, core temperature was measured with a precalibrated rectal probe inserted 6 cm beyond the anus (Tele-Thermometer, Yellow Springs Instruments Co., Yellow Springs, Ohio, USA). Drugs were administered between 10.30 and 11.00 am and at hourly intervals core temperature was measured for 6 h post administration. Drugs used were: naltrexone hydrochloride (Endo Laboratories Inc., Garden City, New York, USA), α-methyldopa (Merck Sharp and Dohme, Haarlem, The Netherlands) and morphine sulphate (O.P.G., Utrecht, The Netherlands). For naltrexone and morphine solutions, 0·9% NaCl (saline) was used as vehicle, α-methyldopa was dissolved in distilled water in the appropriate concentration. Naltrexone or saline was administered subcutaneously (0·1 ml/100 g) 15 min before α-methyldopa or morphine (or vehicle), that was injected intraperitoneally. To provide evidence for an opiate antagonist action of naltrexone experiments were also carried out in which naltrexone was administered

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