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Antagonism of the antidiarrhoeal effect of clonidine and the lethal effect of noradrenaline in rats: a reliable procedure to evaluate the in-vivo α_1- and α_2-blocking activity of drugs?

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Eight compounds with α-adrenergic blocking activity were tested for their ability to antagonize the antidiarrhoeal effect of clonidine (clonidine test) and the lethal effect of noradrenaline (noradrenaline test). Six of the compounds studied are α-adrenergic blocking agents with known α_2/α_1 selectivity. Two compounds, ketanserin (R 41 468) and butanserin (R 53 393), are 5-hydroxytryptamine S_2-antagonists. The ED50-values (mg kg^{-1}) obtained in the clonidine test were: phentolamine (0·34), RX781094 (0·34), yohimbine (0·51), piperoxan (9·36), butanserin (>5·0), prazosin (>10·0), phenoxybenzamine (>40·0), and ketanserin (>80·0). In the noradrenaline test the ED50's (mg kg^{-1}) were: butanserin (0·014), prazosin (0·032), phentolamine (0·59), phenoxybenzamine (1·02), ketanserin (4·69), RX781094 (12·4), piperoxan (21·5), and yohimbine (25·0). The selectivity α_2/α_1-ratios (ED50 clonidine/ED50 noradrenaline were: yohimbine (0·020), RX781094 (0·027), piperoxan (0·44), phentolamine (0·58), ketanserin (>39), prazosin (>312), and butanserin (>357). These results show that yohimbine and RX781094 are equipotent and relatively selective α_2-antagonists; piperoxan and phentolamine block both α_1- and α_2-receptors at closely related doses; ketanserin, prazosin and butanserin are selective blockers of α_1-receptors, ketanserin being very weak, prazosin and butanserin being very potent compounds in this respect. The potent and selective α_1-blocking activity of butanserin, combined to its 5-HT S_2-antagonism makes butanserin a very interesting experimental drug in view of earlier reported data concerning the amplifying effects between 5-hydroxytryptaminergic and noradrenergic vascular mechanisms.

The existence of two subtypes of α-adrenoceptors is well established (for reviews: see Wood et al 1979; Andersson 1981; Langer & Shepperson 1982; Van Zwieten et al 1983; Wikberg 1982). Both receptor subtypes, the so-called α_1- and α_2-adrenoceptors, can be differentiated pharmacologically on the basis of the selectivity by which particular α-adrenergic antagonists block the respective receptors ('Selectivity' here indicates any preference of the test compounds for either α_1- or α_2-receptors without considering interactions with other receptor types). For instance, yohimbine is considered a selective antagonist at α_2-receptors and prazosin a selective α_1-receptor blocker (see below). The objective of the present study was to develop a reliable procedure to assess the α_2/α_1 slectivity of α-blockers in-vivo.

Clonidine, an α_2-adrenergic agonist (Fielding & Lal 1981), has been reported to be a potent anti-diarrhoeal drug in various animal models, e.g. naloxone-precipitated diarrhoea in morphine-dependent rats (Nakaki et al 1981; Shearman et al 1980; Sparber & Meyer 1978), castor oil-induced diarrhoea in rats (Lal et al 1981; Lal & Shearman 1981) and mice (Doherty & Hancock 1983), and prostaglandin E_2-induced diarrhoea in mice (Doherty & Hancock 1983). Clonidine also inhibits normal defaecation (Doherty & Hancock 1983), emotional defaecation (Laverty & Taylor 1969), and decreases small intestinal transit time (Ruwart et al 1980). The effects of clonidine on diarrhoea and intestinal motility are most commonly interpreted as reflecting an effect on α_2-adrenoceptors (Ruwart et al 1980; Nakaki et al 1981; Doherty & Hancock 1983). Indeed, a significant correlation was found between antagonism of the antidiarrhoeal effect of clonidine in-vivo and displacement of clonidine binding in-vitro (Megens et al in preparation) which is related to α_2-receptor binding (U'Prichard et al 1979). Therefore, antagonism of clonidine-induced blockade of castor oil-induced diarrhoea (clonidine antagonism) was selected in the present study to measure the α_2-antagonist activities of drugs.

Antagonism of noradrenaline-induced lethality in rats (noradrenaline antagonism) is a common

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property of α-adrenoceptor antagonists (Niemegeers et al 1977). The potencies of drugs in antagonizing the lethal effects of noradrenaline in rats correlate closely with their potencies in competing for WB 4101 binding sites in-vitro (Peroutka et al 1977), which seems to reflect, in particular, the affinity of drugs towards α_1-receptors (Kapur et al 1979). Consequently, noradrenaline-induced lethality in rats seems to be a specific in-vivo α_1-response and was selected, therefore, to assess the α_1-blocking activities of drugs in the present study.

To determine the reliability of the present procedure, a series of drugs with known α_2/α_1-selectivity were studied: the α_2-selective antagonists yohimbine (Starke & Docherty 1980) and RX781094 (Chapleo et al 1981; Dettmar et al 1981), the mixed α_2/α_1 receptor blockers phentolamine (Borowski et al 1977; Rhodes & Waterfall 1978) and piperoxan (Borowski et al 1977; Blakely & Summers 1978), and the α_1-selective blockers phenoxybenzamine (Drew 1976; Doxey et al 1977) and prazosin (Doxey et al 1977; Starke & Docherty 1980). In addition, two 5-HT S_2-antagonists ketanserin (R 41 468) (Janssen 1982; Leysen et al 1981) and butanserin (R 53 393), a chemical analogue of ketanserin (Leysen et al 1983), were included to test their α_1- and α_2-blocking potential in-vivo.

MATERIALS AND METHODS

Male Wistar rats, 240 to 260 g, were food-deprived during the night before testing. On the day of testing, they were treated subcutaneously with either 0·9% NaCl (saline) or a dose of the test compounds prepared in aqueous solutions (1 ml/100 g). Thirty minutes after treatment, the rats received a subcutaneous injection of clonidine (0·04 mg/10 ml kg^{-1}) and were challenged with 1 ml castor oil orally. Ninety minutes later (120 min after administration of the test compound) the rats were injected with an intravenous dose of noradrenaline (1·25 mg/2 ml kg^{-1}), and the removable floors under the cages were inspected for the presence or the absence of wet, shapeless stools (diarrhoea). Rats surviving 30 min after the noradrenaline dose were considered protected. Two control rats were included in each experiment, the first receiving saline instead of test compound, the second receiving saline instead of test compound and instead of clonidine. Each dose of the test compound was given to 5 rats, and the doses were selected from the geometrical series: 0·005–0·01–0·02 . . . to . . . 20–40–80 mg kg^{-1}. The compounds studied were: butanserin and ketanserin (Janssen Pharmaceutica), phentolamine HCl (CIBA), phenoxybenzamine HCl (SKF), piperoxan HCl (Rhone Poulenc), prazosin HCl (Pfizer), RX781094 HCl (idazoxan) (Reckitt-Colman), yohimbine (Sigma). Clonidine HCl and noradrenaline were obtained from respectively Boehringer and Breon laboratories.

RESULTS

The antidiarrhoeal effect of clonidine

To determine the antidiarrhoeal dose, 40 rats were injected subcutaneously with 4 different doses of clonidine (10 rats per dose) and immediately thereafter challenged with 1 ml castor oil orally. The dose of 0·005 mg kg^{-1} was ineffective, with 0·01 mg kg^{-1} antagonism of diarrhoea was observed in 2, with 0·02 mg kg^{-1} in 8, and with 0·04 mg kg^{-1} in 10 rats. The dose of 0·04 mg kg^{-1}, therefore, was used as a standard dose in all further experiments.

The lethal effect of noradrenaline

The intravenous dose of 1·25 mg kg^{-1} was selected according to previously obtained results in the noradrenaline test (Niemegeers et al 1977).

Control animals

A first control group (n = 60) was injected with saline instead of test compound; 30 min later the rats received clonidine (0·04 mg kg^{-1} s.c.) and 1 ml castor oil orally. None of the rats displayed diarrhoea (normal faecal pellets were observed in 2 animals). The injection of noradrenaline, 90 min later, was lethal in 57 out of 60 rats (95%).

A second control group (n = 60) was injected with saline instead of test compound and instead of clonidine. All these animals displayed diarrhoea; in 58 out of 60 rats (97%) the intravenous noradrenaline injection was lethal.

Experimental animals

Clonidine antagonism. As shown in Fig. 1, there was a dose-related restoration of diarrhoea in clonidine-treated rats with four compounds: yohimbine, RX781094, phentolamine, and piperoxan. Phentolamine, RX781094, and yohimbine were virtually equipotent with ED50 values of 0·34 to 0·51 mg kg^{-1} (Table 1); piperoxan was found to be 28 times less active. By increasing the dose levels, however, clonidine antagonism disappeared (Fig. 1), probably because of the antidiarrhoeal effects of the tested compounds themselves. Indeed, these high doses were found to block castor oil-induced diarrhoea. This disappearance of clonidine antagonism was not observed with phentolamine, which was given in a

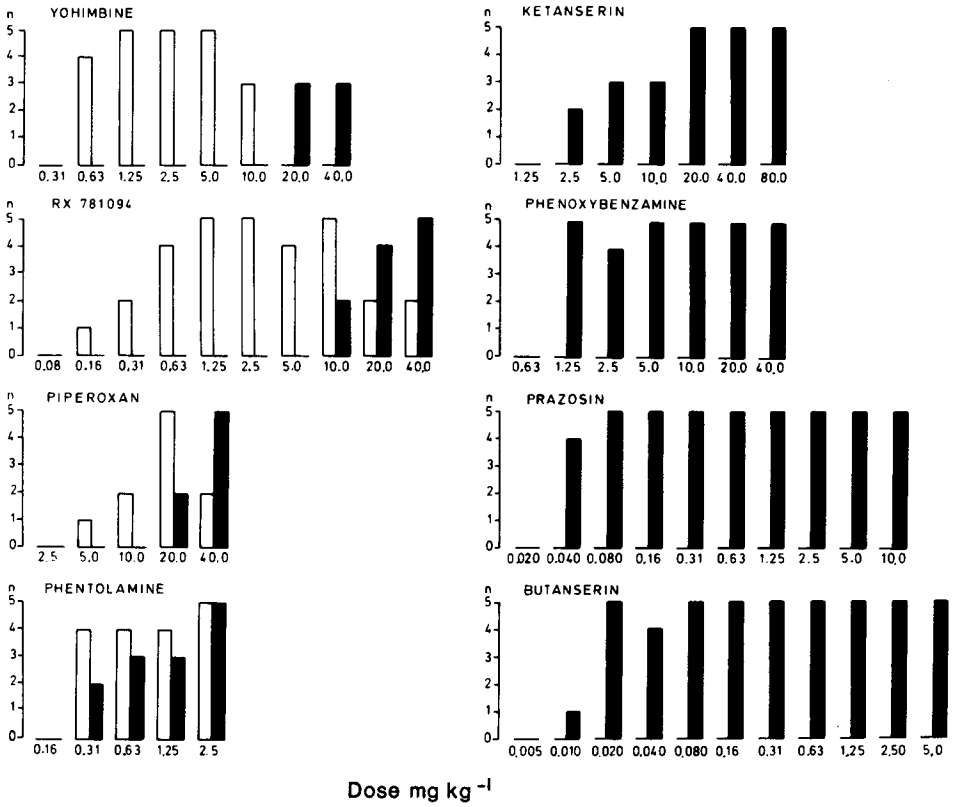

FIG. 1. Number of animals found active in the clonidine test and the noradrenaline test after different doses of the test compounds (n = 5). Solid bars refer to the number of animals surviving the noradrenaline challenge; open bars refer to the number of animals displaying diarrhoea in spite of the clonidine pretreatment. The compounds can be subdivided in three groups: (1) selective antagonists of clonidine (yohimbine, RX781094), (2) antagonists of both clonidine and noradrenaline (piperoxan, phentolamine), (3) selective antagonists of noradrenaline (ketanserin, phenoxybenzamine, prazosin, butanserin).

small dose range only, since the highest dose (2·50 mg kg^{-1}) already completely blocked both the clonidine and the noradrenaline effects. The four other compounds, butanserin, ketanserin, phenoxybenzamine, and prazosin, were devoid of clonidine antagonism up to the highest dose tested. Higher doses of these compounds were not given, either because they antagonized castor oil-induced diarrhoea (ketanserin, phenoxybenzamine) or because the highest dose level tested already exceeded by more than 300 times the ED50-value for noradrenaline antagonism (butanserin, prazosin).

Noradrenaline antagonism. There was a dose-related protection from noradrenaline-induced lethality with all compounds except yohimbine (Fig. 1). With yohimbine the highest dose level given was 40 mg kg^{-1}, because of toxicity symptoms. With the other compounds the ED50 values varied from 0·014 mg kg^{-1} for butanserin to 21·5 mg kg^{-1} for piperoxan (Table 1).

Selectivity. The selectivity ratio was defined as the ratio of the ED50-value obtained in the clonidine test to that obtained in the noradrenaline test. The selectivity ratios are shown in Table 1. They varied from 0·020 for yohimbine to >357 for butanserin. Yohimbine and RX781094 are considered selective antagonists of clonidine since noradrenaline antagonism is only obtained at dose levels respectively 50 and 36 times the anti-clonidine doses. On the other hand, ketanserin, phenoxybenzamine, prazosin and butanserin are considered selective antagonists of noradrenaline since clonidine antagonism was not obtained within the tested dose range. Two compounds, piperoxan and phentolamine, antagonize clonidine and noradrenaline at approximately the same doses (selectivity ratio of about 0·5) and are

Table 1. Activity of the test compounds in the clonidine test and the noradrenaline test in rats.

Compound	Clonidine antagonism ED50 (conf. lim.) mg kg^{-1} s.c. A	Potency	Noradrenaline antagonism ED50 (conf. lim.) mg kg^{-1} s.c. B	Potency	Selectivity ratio A/B
Yohimbine	0·51 (0·38– 0·69)	1·5	25·0 (— - —)[a]	1766	0·020
RX781094	0·34 (0·21– 0·54)	1	12·4 (8·26 –18·5)	884	0·027
Piperoxan	9·36 (6·25–14·0)	28	21·5 (18·2 –33·4)	1535	0·44
Phentolamine	0·34 (0·21– 0·54)	1	0·59 (0·36 – 0·95)	42	0·58
Ketanserin	>80·0	>235	4·69 (2·91 – 7·57)	335	>17
Phenoxybenzamine	>40·0	>118	1·02 (0·68 – 1·53)	73	>39
Prazosin	>10·0	>29	0·032 (0·024– 0·044)	2·3	>312
Butanserin	>5·0	>15	0·014 (0·007– 0·023)	1	>357

[a] Estimated ED50 value; 60% protection was found at 20 and 40 mg kg^{-1}.

thus not considered selective antagonists of either clonidine or noradrenaline.

DISCUSSION

The experimental procedure was developed to evaluate the α_2/α_1 selectivity of test compounds in-vivo. As a prerequisite for this purpose, known specific α_1- or α_2-blockers have to show a clear dissociation in this respect. Based on the selectivity ratios obtained in the present study, the known α-blockers tested can be subdivided in 3 groups: (1) selective antagonists of clonidine (selectivity ratio < 0·1: yohimbine and RX781094), (2) selective antagonists of noradrenaline (selectivity ratio > 10: phenoxybenzamine and prazosin), and (3) non-selective antagonists (0·1 < selectivity ratio < 10: phentolamine and piperoxan).

According to previously reported data (Starke & Docherty 1980; Chapleo et al 1981; Dettmar et al 1981), yohimbine and RX781094 (the drugs that showed an α_2/α_1-selectivity ratio < 0·1) are considered selective α_2-blockers; clonidine antagonism, therefore, seems to be a reliable measure for α_2-blocking activity which is in agreement with the α_2-character of the antidiarrhoeal effect of clonidine (Ruwart et al 1980; Nakaki et al 1981; Doherty & Hancock 1983). Furthermore, data from our own laboratories indicate a significant correlation between displacement of clonidine binding in-vitro and antagonism of the antidiarrhoeal effect of clonidine in-vivo (Megens et al to be published).

Prazosin and to a lesser degree phenoxybenzamine (the compounds displaying a α_2/α_1-selectivity ratio >10) have been reported to be α_1-selective compounds (Drew 1976; Doxey et al 1977; Starke & Docherty 1980). Noradrenaline antagonism, therefore, seems to be indicative for α_1-blocking activity, which is on line with the close correlation between the potencies of drugs in antagonizing noradrenaline-induced lethality and their potencies in competing for α_1-binding sites labelled with WB4101 in-vitro (Peroutka et al 1977).

Phentolamine and piperoxan (test compounds which showed an α_2/α_1-selectivity ratio between 0·1 and 10) are generally considered mixed α_2/α_1 antagonists (Borowski et al 1977; Blakely & Summers 1978; Rhodes & Waterfall 1978). Indeed, both compounds have been found to antagonize clonidine and noradrenaline in about the same dose range. In view of these data, it can be stated that the present procedure is a rapid and reliable method to study the in-vivo dissociation between the α_1- and α_2-blocking activities of drugs.

Apart from the above α-blocking drugs, two 5-HT S_2-antagonists, ketanserin and butanserin, were included to test their α_1- and α_2-blocking activity in-vivo. Both compounds were found to be devoid of α_2-blocking activity since they did not antagonize the antidiarrhoeal effect of clonidine in the dose range tested. On the other hand, the two compounds did antagonize the lethal effect of noradrenaline. These observations suggest that both compounds are selective blockers at α_1-adrenoceptors, ketanserin being a weak, and butanserin being a very potent, compound in this respect. These in-vivo results completely agree with the binding profiles of both compounds in-vitro (Leysen et al 1981, 1983). Anti-5-HT activity as measured by antagonism of tryptamine-induced cyanosis (Niemegeers, unpublished results) is reached at a dose of 0·056 mg kg^{-1} for ketanserin and at 0·074 mg kg^{-1} for butanserin. In other words, ketanserin is in the first place a 5-HT antagonist with a dissociation > 80 between α_1-blocking activity and anti-5HT- activity. Butanserin, on the other hand, is an antagonist of both 5-HT and noradrenaline since

the dissociation between both activities is only 5. The potent and selective α_1-blocking activity of butanserin, combined with its 5-HT S_2-antagonism makes butanserin a very interesting experimental drug in view of the earlier reported data concerning the amplifying effects between 5-hydroxytryptaminergic and noradrenergic vascular systems (cf. Van Nueten et al 1981).

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