Naltrexone and the behavioural action of clonidine in normotensive and spontaneously hypertensive rats

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Abstract—The present experiments were aimed at the behavioural effects of clonidine and the possible involvement of opiate systems therein. Previous work had shown that opiate antagonists could block the effects of clonidine on blood pressure. In the open-field, clonidine treatment induced a decrease in ambulation and rearing activity. This effect was similar in normotensive Wistar and Wistar-Kyoto rats and in spontaneously hypertensive rats. In neither strain did naltrexone pretreatment influence the behavioural action of clonidine, however. These results do not lend support to the proposed interaction between opiate systems and the central effects of clonidine.

Clonidine is an imidazoline derivate which has been widely used as an antihypertensive agent. Its action was shown to be exerted by central mechanisms, with the site being located in the medulla oblongata (Laubie & Schmitt 1977; Kobinger 1978; Korner & Angus 1981). Clonidine is an α_2-adrenoreceptor agonist, and its pharmacological action could be mediated either by presynaptic inhibition of central noradrenergic systems or by direct activation of central postsynaptic adrenoceptors (Drew et al 1979; Head et al 1983; Van Zwieten & Timmermans 1983; Kalsner 1985). In spontaneously hypertensive rats (SHR) clonidine may cause a marked decrease in blood pressure, while being virtually ineffective in normotensive Wistar-Kyoto controls (WKY) (Head & De Jong 1986). In SHR the levels and turnover of noradrenaline in various medullary sites have been shown to be changed (Versteeg et al 1984).

Evidence is now accumulating that the mechanism of action of clonidine may also involve central opiate systems. Thus, in SHR its antihypertensive action could be inhibited by pretreatment with the opiate receptor antagonists naloxone or naltrexone (Farsang & Kunos 1979; Kunos et al 1987). In-vitro, clonidine was able to induce the release of an endorphin-like substance from medullary tissue of SHR, but not WKY rats (Kunos et al 1981).

Not only does clonidine cause changes in blood pressure, it also affects behaviour. In rats, it is a sedative, inducing a decrease in arousal and locomotor activity (Drew et al 1979; Leaton & Cassella 1984; De Sarro et al 1987). Treatment with clonidine furthermore induced analgesia (Paalzow & Paalzow 1976; Kunos et al 1987). In this respect it is interesting to note that SHR show behavioural hyperreactivity (Tilson et al 1977; Van den Buuse et al 1984) as well as a naloxone-reversible decrease in pain-sensitivity (Sitsen & De Jong 1984; Kunos et al 1987).

Little is known on the relative effect of clonidine on behaviour in hypertensive and normotensive subjects and on the possible involvement of central opiate systems in this action. Therefore, we studied the effect of clonidine on open-field behaviour in SHR, WKY and Wistar rats, and furthermore investigated whether pretreatment with naltrexone could antagonize these effects.

Materials and methods

Male rats were used. They were either regular Wistars, WKY or

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SHR, and were derived from a breeding colony at our institute. The rats were kept 4–5 in a cage at a constant light-dark rhythm with standard pellet food and tap water freely available. At the time of the experiments the rats were 7–9 weeks old, and 180–220 g.

Open-field behaviour was measured as previously described (van den Buuse et al 1984). Briefly, behaviour was scored during 5 min after placement of the rat in the centre of an 80 cm diameter open-field. Ambulation was scored as the number of crossings of floor units. For this, the floor of the open-field was divided into units by two concentric circles and a number of radial lines. Rearing was scored when the animal stood stretched on its hind-paws. Grooming was scored in bouts of 5 s.

Clonidine was injected subcutaneously and naltrexone was injected intraperitoneally (i.p.). The substances were given on a weight/body weight basis, with the injection volume being 1 mL kg^{-1}. Clonidine was injected 20 min before the open-field test. Naltrexone was injected 40 min before the tests, thus 20 min before clonidine. Control injections consisted of equal volumes of 0.9% saline. Rats were used once.

Experimental data were analysed with analysis of variance (ANOVA), in appropriate cases followed by Student-Newman-Keuls test for multiple comparisons. ANOVA's are described in the Results-section, multiple comparison is shown in the tables. Differences were considered to be significant when $P < 0.05$.

Results

The effect of different doses of clonidine (10–300 μg kg^{-1}) on open-field behaviour of Wistar, WKY or SHR is shown in Table 1. In all strains clonidine induced a decrease in ambulation. This was shown by a significant overall effect of dose [$F(3,5) = 47.3$, $P < 0.001$] but the absence of a significant interaction between dose and strain. A significant overall effect of strain was observed [$F(2,5) = 29.3$, $P = 0.004$]. This strain-difference was especially prominent between Wistar and WKY and between WKY and SHR.

Also, rearing score was decreased by clonidine (Table 1), as shown by a significant effect of dose [$F(3,5) = 23.9$, $P < 0.001$]. A significant overall effect of strain [$F(2,5) = 32.2$, $P < 0.001$] was found, reflecting the markedly higher rearing scores in SHR and, to a lesser extent, in Wistar rats. The decrease in rearing after clonidine was most marked in SHR and, to a lesser extent, in Wistar animals. This resulted in a significant overall interaction between strain and dose for rearing [$F(7,6) = 3.6$, $P = 0.002$].

Grooming behaviour was inhibited by clonidine [effect of dose: $F(4,6) = 6.5$, $P < 0.001$]. Although no overall strain-difference was observed, a significant interaction of strain and dose [$F(7,6) = 4.1$, $P < 0.001$] indicated differential effects of clonidine between the strains. This finding is probably caused by the decrease in grooming score in Wistar rats, the increase followed by a decrease in WKY, and the relative absence of an effect in SHR (Table 1).

The effect of pretreatment with naltrexone (1 mg kg^{-1} i.p.) on the behavioural action of clonidine is shown in Table 2. The dose of clonidine was chosen from the results of the experiments shown in Table 1. The dose of naltrexone was chosen from

Table 1. The effect of increasing doses of clonidine (10–300 µg kg⁻¹) on open-field behaviour of Wistar, WKY or SHR.

Drug and dose (µg kg⁻¹)	Wistar	WKY	SHR	
Ambulation				
Saline	114·7±5·3	103·0±3·1	112·7±4·2	
Clonidine 10	n.m.	99·7±9·4	116·7±5·9	
Clonidine 30	87·7±6·3	74·3±9·7*	83·3±7·5*	
Clonidine 100	67·7±7·1*	36·0±9·9*	50·8±6·9*	(a)
Clonidine 300	38·3±9·0*	14·8±9·7*	25·8±9·9*	
Rearing				
Saline	19·8±3·5	6·8±0·7	26·1±2·8	(a,c)
Clonidine 10	n.m.	6·4±1·8	25·6±2·7	(c)
Clonidine 30	15·8±3·3	4·8±1·5	22·8±4·3	(a,b,c)
Clonidine 100	9·7±1·2*	2·0±0·9	7·5±1·4*	
Clonidine 300	3·3±1·7*	1·0±0·6	4·7±2·4*	
Grooming				
Saline	7·5±2·4	2·6±0·4	3·3±0·7	(a,b)
Clonidine 10	n.m.	3·6±1·0	3·9±0·6	
Clonidine 30	4·0±1·1	7·8±1·4	3·0±0·7	(c)
Clonidine 100	4·3±1·5	4·2±0·8	3·3±0·5	
Clonidine 300	0*	0·5±0·2	2·2±1·0	

*$P < 0.05$ for difference with saline group of the same strain.
(a) $P < 0.05$ for difference between Wistar and WKY for this treatment.
(b) $P < 0.05$ for difference between Wistar and SHR for this treatment.
(c) $P < 0.05$ for difference between WKY and SHR for this treatment.

preliminary experiments (not shown) and from literature data. For ambulation, an effect of strain was found [$F(2,4) = 23·2$, $P < 0·001$] and an effect of clonidine [$F(1,4) = 169·0$, $P < 0·001$]. Naltrexone by itself did not produce a significant overall effect on ambulation, but a significant interaction between clonidine and naltrexone was found [$F(1,5) = 9·3$, $P = 0·003$]. This interaction is probably caused by the slight decrease in ambulation scores in WKY and SHR after naltrexone, but any further net decrease in ambulation in rats treated with naltrexone and clonidine compared with those administered clonidine was not observed. Thus, naltrexone did not affect the clonidine-induced decrease in ambulation score in either of the strains (Table 2).

Table 2. The effect of pretreatment with naltrexone (1 mg kg⁻¹) on the behavioural action of clonidine (50 µg kg⁻¹) in the open-field.

t = −40 min t = −20 min	Saline Saline	Naltrexone Saline	Saline Clonidine	Naltrexone Clonidine
Ambulation				
Wistar	128·1±6·7	125·5±8·1	89·9±5·3*	102·5±8·7*,**
WKY	116·9±3·8	90·3±9·9*	56·5±4·8*	56·5±8·6*,**
SHR	133·8±5·5	115·2±8·6	71·5±2·9*	77·0±3·1*,**
		(a,c)	(a)	(a)
Rearing				
Wistar	15·2±1·9	15·2±2·7	9·0±1·1	8·7±1·8
WKY	7·9±1·1	5·8±1·4	4·5±0·9	3·0±0·7
SHR	27·5±2·4	25·8±2·6	12·6±2·1*	10·5±1·4*,**
	(a,b,c)	(a,b,c)	(c)	
Grooming				
Wistar	4·1±1·3	5·8±1·3	3·9±0·6	4·7±0·3
WKY	5·7±1·3	4·3±0·7	6·1±1·3	2·7±0·9
SHR	2·2±0·6	4·8±1·6	6·2±0·9	5·8±0·8

*$P < 0·05$ for differences with saline/saline group of the same strain.
**$P < 0·05$ for difference with naltrexone/saline group of this strain.
(a) $P < 0·05$ for difference between Wistar and WKY for this treatment.
(b) $P < 0·05$ for difference between Wistar and SHR for this treatment.
(c) $P < 0·05$ for difference between WKY and SHR for this treatment.

Analysis of rearing scores yielded a significant effect of strain [$F(2,4) = 61·2$, $P < 0·001$] and of clonidine [$F(1,4) = 62·3$, $P < 0·001$] but not of naltrexone. Moreover, the absence of an interaction between naltrexone and clonidine showed that the pretreatment of rats with naltrexone did not influence the effect of clonidine on open-field rearing activity. As in the first experiment, an interaction between strain and clonidine [$F(2,5) = 11·9$, $P < 0·001$] indicated the greater clonidine-induced decrease in this score in SHR and wistar, as opposed to WKY rats.

Grooming scores did not show any overall effects, but a significant interaction between strain and naltrexone [$F(2,5) = 3·5$, $P = 0·036$] was found. This latter finding may represent differential effects of naltrexone-treatment on grooming between the strains.

Discussion

The present experiments were aimed at the behavioural effects of clonidine treatment and the possible involvement of opiate systems therein. Clonidine induced a decrease in open-field activity, as shown by lower scores for ambulation and rearing. Pretreatment with naltrexone was without influence on this effect.

Central and peripheral administration of low doses of clonidine has been shown to induce a marked decrease in blood pressure, especially in hypertensive subjects (Korner & Angus 1981; Head & De Jong 1986). Some reports have shown that this effect can be inhibited by pretreatment with naltrexone, thus suggesting the involvement of opiate systems (Farsang & Kunos 1979; Kunos et al 1987). Our results do not suggest a significant involvement of opiates in the behavioural action of clonidine, since naltrexone did not influence its behavioural effects. A number of points can be raised on this issue, however. Firstly, it is clear that higher doses of clonidine are needed to obtain a decrease in open-field activity than are needed to induce a decrease in blood pressure (Head & De Jong 1985). It could be that the present effects are caused by a general sedation, rather than by a specific effect on arousal or fear. Even then opiates could play a role in the action of clonidine. The high dose of clonidine needed to produce behavioural effects in the present experiments could, nevertheless, mask any possible antagonism of opioid activity by naltrexone. It has been shown that naloxone pretreatment was unable to antagonize the cardiovascular effect of relatively high doses of clonidine (Head & De Jong 1985). Moreover, the blood pressure decrease caused by α-methyldopa, an indirect noradrenergic agonist, could be antagonized by naltrexone only when low doses were used (Van Giersbergen & De Jong 1988). Increasing the dose of α-methyldopa increased the blood pressure fall, but diminished the inhibiting effect of naltrexone pretreatment (Van Giersbergen & De Jong 1988).

Another consideration could be that the behavioural effects of clonidine are mediated by central processes which do not involve opiates. In this respect it is interesting that the behavioural effect of clonidine was recently localized in the locus coeruleus, and could thus be a pure noradrenergic effect (De Sarro et al 1987). The nucleus tractus solitarius appears to be the site of action for the cardiovascular effects of clonidine (Zandberg & De Jong 1977; Petty & De Jong 1984; Versteeg et al 1984). Local injection of α-adrenoceptor agonists in this nucleus induced a decrease in blood pressure which could be antagonized by opiate receptor blockers or antisera against endorphins (Petty & De Jong 1984; Kunos et al 1987).

In SHR, clonidine has been shown to induce a greater decrease in spontaneous activity than in WKY (Tilson et al 1977). Also in other tests, SHR were found to be more sensitive to clonidine than WKY (Eriksson et al 1980; Yarbrough et al

1983). In our experiments, clonidine exerted comparable effects on open-field activity of SHR and WKY, however. The reason for this discrepancy is unclear, but could be the result of differences in the experimental paradigm. Thus, in our experiments other noradrenergic pathways could have been involved, which showed similar sensitivity to clonidine in the three strains.

In conclusion, the present results show that clonidine may induce a decrease in behavioural activity. These effects are not influenced by pretreatment with an opiate antagonist. Thus, an involvement of opiates in the behavioural effects of clonidine appears unlikely.

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