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The Role of Central Postsynaptic α_2-Adrenoceptor on the Immobility Duration in the Forced-swimming Test Mice

Byung-Yong Rhim, Sang-Kon Kim, Won-Suk Lee and Ki-Whan Hong

Department of Pharmacology, College of Medicine, Pusan National University, Pusan 600, Korea

ABSTRACT

1) In the study of the forced-swimming test in mice (FSM), the duration of immobility posture was dose-dependently shortened by α_2-agonists, clonidine and guanabenz. BH-T 933 and oxymetazoline also decreased it. Xylazine rather increased the immobility duration at low dose.

2) α_1-Agonists, cirazoline, amidephrine and methoxamine, however, showed inconsistent effect on the immobility duration (ID).

3) The decrease in ID by clonidine and guanabenz was antagonized by pretreatment with yohimbine, idazoxan and phentolamine ($\alpha_2$antagonist), but not by prazosin and corynanthine (α_1-antagonist).

4) The ID in the FSM was shortened dose-dependently by *d*-amphetamine, and it was also antagonized by yohimbine, but not by prazosin.

5) In the mice pretreated with either α-methyl-*p*-tyrosine or reserpine, or with combination of both, the decrease in ID was still evoked by clonidine.

6) When the mice were chronically treated with antidepressants (desipramine and imipramine), or with electroconvulsive shock, clonidine still decreased the ID as it did in the control.

7) These results provided the evidences to hypothesize that the change of the ID in the FSM is closely related with the postsynaptic α_2-adrenoceptor located on the central noradrenergic neuron body. Furthermore, it is assumed that this escape-directed behavior enhanced by α_2-adrenoceptor agonist may be the result in some analogy with the incentive of drives which are directed toward the self-preservation.

Abbreviation: ID; immobility duration, FSM; the forced-swimming test in mice

INTRODUCTION

As a novel behavioral model of depression a forced-swimming test in rats has been described by Porsolt *et al.* (1977, 1978). The procedure is based on the observations that rats or mice when forced to swim in the water-filled restricted vessel take a characteristic immobile posture after swimming around for a while. Meanwhile the duration of immobility is measured. The immobility duration itself was estimated as a state of lowered mood or hopelessness in animals, as it was reduced in the animals treated with antidepressants.

Nevertheless, besides tricyclic antidepressants, anticholinergic drugs (Browne, 1979), antihistamines (Wallach & Hedley, 1979), serotonin antagonists (Luttinger *et al.*, 1985) and non-pharmacological

treatments such as electroconvulsive shock and REM sleep deprivation (Porsolt *et al.*, 1978) have been reported to reduce the immobility. The results of these non-specificity have raised the question whether the reduction in immobility in this forced-swimming test is really useful model for estimating antidepression and screening antidepressant drugs (Browne, 1979; Schechter & Chance, 1979).

In the present investigation, the interests are focused on the escape-directed behavior oppositely rather than on the immobility aspect. In view of the facts that when animals are forced to swim in a restricted space, they act on instinct to escape from the vessel, this behavior is considered apparently to coincide with drives, in that they initiate random behaviors until learning (Valzelli, 1981), and must be discriminated from exploratory activity. Under the working hypothesis that the escape-directed behavior is instinctive behavior reflexly evoked, the characterizations of the this behavior were pharmacologically analyzed by determining the change of immobility duration antagonists. The results were discussed in reference to the role of central α_2-adrenoceptor in relation with the instinctive behavior of the animals.

MATERIALS AND METHODS

Mice weighing 25-35 g of either sex were used. Upon arrival the mice were housed 10/cage with food and tap water available *ad libitum* for at least 1 week prior to behavioral testing. Briefly, the procedure was basically the same as that described by Porsolt (1981). The animals were plunged into the plexiglass cylinder (height: 25 cm; diameter: 10 cm) filled with water (23°C) up to 6 cm in height. As a preliminary learning, the animals were trained swimming for 15 min, and thereafter, on a second exposure after 24 hours, the duration of immobile posture that they took in 5-min schedule was cumulatively measured in seconds.

Immobility was defined as the minimal movement necessary for the mouse to stay float. All the manipulation was conducted between 10 and 15 o'clock. Prior to every experiment using drugs, the control mean level of 6 animals was measured for calculation of percent change. In the control group physiological saline (0.1 ml/10 g) was intraperitoneally administered instead of drugs. Unless stated otherwise, the animals were allowed to leave in the cage for 60 min after the administration of agonists until the forced-swimming test, and every antagonist was administered 15 min prior to agonist injection. Imipramine and desipramine were administered intraperitoneally with a dose of 20 mg/kg once daily for 14 days, respectively. Electroconvulsive shock was applied once daily for 10 days using the Reither Model SOS ECT unit by delivering AC (90 V, 60 Hz) for 1 sec through ear clip electrodes under light ether anesthesia.

The drugs used were clonidine HCl (Tokyo Chem.), guanabenz acetate (Wyeth), B-HT 933 (Boehringer-Ingelheim), xylazine (Bayer), oxymetazoline HCl (Schering), yohimbine HCl (Sigma), idazoxan (RX 781094, Reckitt an Colman), phentolamine HCl (Ciba-Geigy), cirazoline HCl (Synthelabo), methoxamine (Burroughs Wellcome), amidephrine mesylate (Sigma), prazosin HCl (Pfizer), corynanthine HCl (Sigma), *d*-amphetamine sulfate (Sigma), reserpine (Sigma), α-methyl-*p*-tyrosine (Sigma), imipramine HCl (Yung Pung Co.) and desipramine HCl (Revlon Health Care Group).

Statistical analysis: The immobility duration of the various treatment groups is expressed as a percent of the mean of the control. Statistical comparisons were performed using t-test.

RESULTS

In pilot study the immobility duration was gradually increased when the trial of forced-swimming test was repeated according to the time course. On a second exposure of test after 24 hours the immobility duration was 141.8 ± 8.2 sec. When the same procedures were repeated in 1, 2 and 3 weeks, the immobility duration was gradually lengthened by 184.1 ± 13.9, 199.3 ± 10.2 and 228.5 ± 11.3 sec,

Table 1. Effect of α_2-adrenoceptor agonists on the immobility duration in the forced-swimming mice

α_2-Agonists	Dose (μg/kg)	n[a]	% of control	P value
Clonidine	10	6	72.4± 3.7	<0.001
	100	26	44.6± 4.3	<0.001
	500	10	28.92 3.5	<0.001
	1000	11	15.3± 4.7	<0.001
Guanabenz	100	11	60.5± 8.5	<0.001
	500	12	43.7± 6.4	<0.001
	1000	8	22.3± 8.0	<0.001
Oxymetazoline	500	7	62.6± 13.3	<0.05
	1000	6	101.8± 9.1	NS[b]
Xylazine	100	7	129.9± 8.4	<0.05
	500	6	66.7± 13.7	NS
	1000	7	98.5± 10.1	NS
B-HT 933	100	6	126.8± 9.0	<0.05
	500	7	97.6± 16.7	NS
	1000	6	75.5± 8.3	<0.05

a: n represents the number of experiments.

b: NS means no significance.

Table 2. Effect of α_1-adrenoceptor agonists on the immobility duration in the forced-swimming mice

Drugs	Dose (mg/kg)	n[a]	% of control	P value
Cirazoline	0.5	15	81.0±4.2	NS[b]
Amidephrine	0.5	5	82.3±5.9	NS
Methoxamine	1.0	8	133.9±9.0	NS

a: n represents the number of experiments.

b: NS means no significance.

respectively, and thereafter remained constant. With these results it was concluded that the forced-swimming test might be managed at 24-hour after the first exposure for training.

Effect of α-adrenoceptor agonists

Table 1 shows the effect of α_2-adrenoceptor agonists. Both clonidine (10-1,000 μg/kg) and guanabenz (100-1,000 μg/kg) dose-dependently inhibited the immobility duration in forced-swimming mice, and oxymetazoline (500 μg/kg) and B-HT 933 (1,000 μg/kg) showed nondose-dependent inhibition. However, xylazine did rather increase the immobility duration at lower dose (100 μg/kg).

On the other hand, α_1-adrenoceptor agonists, cirazoline, amidephrine and methoxamine exerted little effect on the immobility duration (Table 2).

Effect of α-adrenoceptor antagonists

As demonstrated in Table 3, the immobility duration was significantly lengthened by α_2-adrenoceptor

Table 3. Effect of α_1-and α_2-antagonists on the immobility in the forced-swimming mice

Antagonists	Dose (mg/kg)	n[a]	% of control	P value
α_1-Antagonist				
Prazosin	0.1	16	81.0± 5.6	NS[b]
Corynanthine	0.5	6	112.3± 3.9	NS
α_2-Antagonist				
Yohimbine	1.0	6	120.7±10.3	NS
	5.0	13	139.8± 6.8	<0.001
Idazoxan	1.0	7	126.7± 7.5	<0.05
	5.0	7	156.8± 6.4	<0.001
Phentolamine	1.0	9	71.0± 8.4	<0.001

a: n represents the number of experiments.

b: NS means no significance.

Table 4. Inhibitory effect of α-antagonists on the clonidine or guanabenz-induced decrease in immobility in the forced-swimming mice

Antagonist,	mg/kg	α_2-Agonist,	μg/kg	n[a]	% of control	P value
α_2-Antagonists						
Yohimbine,	5.0	Clonidine,	100	17	119.1± 7.8	<0.05
		Guanabenz,	100	5	103.7± 5.7	NS[b]
		Guanabenz,	1000	8	108.5±11.6	NS
Idazoxan,	5.0	Clonidine,	100	13	129.8± 5.2	<0.001
		Guanabenz,	500	5	95.1±16.3	NS
		Guanabenz,	1000	6	111.4± 2.8	<0.01
Phentolamine,	1.0	Clonidine,	100	7	98.0± 8.1	NS
α_1-Antagonists						
Prazosin,	0.5	Clonidine,	100	17	32.7± 7.3	<0.001
Corynanthine,	5.0	clonidine,	500	6	45.22 9.8	<0.01

a: n represents the number of experiments.

b: NS means no significance.

antagonists, yohimbine and idazoxan, but not by α_1-antagonists, prazosin and corynanthine with the exception of phentolamine, which exerted the decrease of immobility duration.

Effect of α-antagonist pretreatment

The immobility duration which was shortened by clonidine or guanabenz was selectively blocked or rather prolonged by the pretreatment with yohimbine, idazoxan or phentolamine. On these actions α_1-antagonist, prazosin or corynanthine did not affect as shown in Table 4.

Table 5. Effect of α-antagonist on the *d*-amphetamine-induced decrease in immobility in the forcedswimming mice

Antagonist (mg/kg)	*d*-Amphetamine (mg/kg)	n[a]	% of control	P value
Non-treatment	0.5	7	73.3 ± 14.6	NS[b]
	2.0	10	45.3 ± 11.7	<0.01
	3.0	11	43.7 ± 7.1	<0.001
	5.0	12	7.7 ± 2.3	<0.001
Prazosin, 0.1	3.0	11	30.0 ± 9.3	<0.001
Yohimbine, 5.0	3.0	5	98.2 ± 4.2	NS

a: n represents the number of experiments.

b: NS means no significance.

Table 6. Effect of pretreatment of either α-methyl-*p*-tyrosine (α-MPT) or reserpine and their combination on the clonidine induced-immobility in forced-swimming mice

Drugs, mg/kg	Agonist, μg/kg	n[a]	% of control	P value
α-MPT, 200	Saline	7	103.5 ± 4.4	NS[b]
	Clonidine, 100	7	74.5 ± 9.9	<0.05
Reserpine, 2.0	Saline	7	90.0 ± 8.0	NS
	Clonidine, 100	7	42.8 ± 6.4	<0.001
Reserpine, 2.0	Saline	6	93.3 ± 3.2	NS
+ α-MPT, 200	Clonidine, 100	6	57.2 ± 5.2	<0.001

a: n represents the number of experiments.

b: NS means no significance.

Action of d-amphetamine

This experiment was aimed to assess the effect of α-antagonist on the *d*-amphetamine action, decreasing the immobility duration in the forced-swimming test. *d*-Amphetamine exerted decrease in immobility duration in a dose-dependent manner with the dose range between 0.5 and 5.0 mg/kg. The pretreatment with yohimbine (5.0 mg/kg) completely blocked the decrease in immobility duration by *d*-amphetamine, whereas prazosin (0.1 mg/kg) did not elicit the antagonism (Table 5).

Effect of catecholamine depletion

To elucidate the action site of α_2-agonist in the central noradrenergic neurons, this experiment was conducted. The animals were pretreated with α-methyl-*p*-tyrosine (200 mg/kg 4 hours prior to experiment) or reserpine (2 mg/kg, 24 hours). As shown in Table 6, the immobility duration was little altered by pretreatment with α-methyl-*p*-tyrosine, reserpine or their combination. Nevertheless, clonidine administration on these mice deprived of catecholamine exerted the significant decrease in the immobility duration.

Table 7. Effect of pretreatment of either antidepressants or electro-convulsive shock on clonidine-induced immobility in forced-swimming mice

Pretreatments	(mg/kg)	Drug	(μg/kg)	n[a]	% of control	P value
Chronic						
Imipramine,	20	Saline		5	91.2± 7.7	NS[b]
		Clonidine,	100	3	47.2±12.2	<0.05
Desipramine,	20	Saline		6	80.5± 6.0	<0.05
		Clonidine,	100	5	52.1± 9.2	<0.01
Electroconvulsive		Saline		8	92.1± 4.4	NS
shock		Clonidine,	100	7	62.3± 7.8	<0.01

a: n represents the number of experiments.

b: NS means no significance.

Effects of chronic tricyclic antidepressant and electroconvulsive shock

This experiment was aimed to discriminate the acute effect of clonidine from that of chronic treatment with antidepressants or electroconvulsive shock on the immobility duration. Chronic administration of desipramine (with a dose of 20 mg/kg i.p. once daily for 14 days), decreased the immobility duration by 20% ($p<0.05$), whereas that of imipramine (the same dose) or electroconvulsive shock did little. The administration schedule in this experiment was different from other reports (Porsolt, 1981; Satoh *et al.*, 1984). Even on these animals, clonidine administration in addition caused significant decrease in the immobility duration irrespective of the pretreatment with antidepressants or electroconvulsive shock (Table 7).

DISCUSSION

Many reports have emphasized the immobility itself as a model of clinical depression with the evidences that antidepressants or electroconvulsive shock produced a reduction in the duration of immobility (Porsolt *et al.*, 1977;1978). Nevertheless, some controversials are raised. They usually gave the antidepressants in 3 i.p. injection schedule, 24, 5, and 1 h before the test (Porsolt *et al.*, 1977;1978;Araki *et al.*, 1984; Satoh *et al.*, 1984). However, it is widely known that the induction of therapeutic effects of antidepressants requires the prolonged treatment for more than two weeks (Oswald *et al.*, 1972; Klein *et al.*, 1980). In this experiment, chronic imipramine or electroconvulsive shock treatment for 14 days did not evoke the significant reduction of immobility, but desipramine-treatment induced the reduction of immobility. This indicates the inconsistent results of antidepressant action on this system. Moreover, besides tricyclic antidepressants, anticholinergics, antihistamines, pentobarbital and triiodothyronine etc. that have no effect on depression are demonstrated to induce the reduction of immobility (Browne, 1979; Wallach & Hedley, 1979; Schechter & Chance, 1979). In view of these considerations, the interests are focused on the behavior of escape-directed aspects rather than the immobility itself. The animals placed in the water exhibit definitely the behavior to escape during the first 2 min and thereafter, intermittently during the last 3 min. These behaviors were estimated as acts on instict to escape from the vessel. When these activites are being directed toward incentives the drives coinciding with instincts arise without motivation (Valzelli, 1981). Such activites are usually blind so that they initiate random behavior. This behavior acting on instinct is termed as escape-directed behavior according to Kitada *et al.* (1981).

Of particular interest for the mechanisms involved in the incentives of drives are the effect of α_2-adrenoceptor agonists and the antagonism by α_2-adrenoceptor antagonist, yohimbine, idazoxane and phentolamine on the agonist actions (Shepperson *et al.*, 1981; Chapleo *et al.*, 1981; Timmermans *et al.*, 1984; Weiner, 1980), but not by α_1-adrenoceptor antagonist, prazosin or corynanthine. Furthermore, clonidine still yielded the apparent reduction of immobility even after combined treatment with α-methyl-*p*-tyrosine and reserpine.

These results have provided the evidence that this escape-directed behavioral response is mediated through stimulation of central postsynaptic α_2-adrenoceptor. In addition, this speculation is further supported by the fact that after chronic treatment with antidepressants or electroconvulsive shock, clonidine can still evoke the reduction of immobility. Recently, many studies have demonstrated that chronic antidepressant treatment is associated with a decrease in the number of presynaptic α_2-adrenoceptor binding sites, resulting in the subsensitivity of this receptor (Svensson & Usdin, 1978; Smith *et al.*, 1981; McMillen *et al.*, 1980; Campbell *et al.*, 1982). Therefore, it is concluded that clonidine must act on the postsynaptic site.

One question that can be raised is why xylazine can not lead to the reduction of immobility in the forced-swimming mice. Xylazine is known to have a high partition coefficiency (Summers *et al.*, 1980) and to induce depression in chicks as well as clonidine and guanabenz, the action of which is selectively blocked by α_2-adrenoceptor antagonists (Hsu, 1981; Roach *et al.*, 1983). Nevertheless, xylazine failed to reduce the immobility but rather prolonged it instead. The present findings seem to suggest that the immobility behavior is little analogy with the clinical depression in mice. The other question is whether the dose-dependent reduction of immobility by clonidine or guanabenz is mediated by the stimulation of postsynaptic α_1-adrenoceptor as demonstrated by Anden *et al.*, (1976) and Nishikawa *et al.* (1983). It seems unlikly since prazosin or corynanthine failed to reverse the immobility duration.

On the other hand, Kitada *et al.* (1981) have suggested that metamphetamine yielded different kind of behavior from that evoked by antidepressants, as the behavior by the former is ascribed to general motor activity. However, it is assumed practically difficult to differentiate the escape-directed behavior from general motor activity in the forced swimming test. Of interesting findings were the antagonism by yohimbine on the *d*-amphetamine-induced reduction of immobility and prazosin also failed to block it. It seems likly that *d*-amphetamine may cause the reduction of imobility by releasing noradrenaline from central noradrenergic neurons as an indirect-acting sympathomimetics.

Establishment of more accurate definition of drives on instict and assessment of neurochemical correlation with its behavior may provide further insights into the methodology to approach clinical depression with antidepressants.

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=국문초록=

새앙쥐 강제수영시 부동자세 시간에 대한 Central postsynaptic α^2-Adrenoceptor의 역할에 대한 연구

부산대학교 의과대학 약리학 교실

임병용, 김상곤, 이원식, 홍기환

새앙쥐 강제수영 실험에서 부동자세 시간의 단축은 향주도성 행동의 항진이라는 본능적 충동의 유발이라는 가정 아래 중추 noradrenaline neuron에 있어서 α_1- 및 α_2-adrenoceptor의 역할과 관련지어 이 실험을 행하였고 다음과 같은 결론을 얻었다.

1. 새앙쥐를 이용한 강제수영 실험에서 부동자세 시간은, clonidine 및 guanabenz 등과 같은 α_2-agonists에 의하여 용량에 의존하여 단축되었다. B-HT 933 및 oxymetazoline은 용량에 의존하지 않으나 단축시켰다. xylazine에의하여는 오히려 증가되었다.
2. α_1-Agonists 인 cirazoline, amidephrine 및 methoxamine은 부동자세 시간에 일관성 있는 영향을 미치지 아니하였다.
3. Clonidine과 guanabenz에 의한 부동자세 시간의 단축은 α_2-antagonists, yohimbine, idazoxan 및 phentolamine 전처치로 봉쇄되었으나 α_1-antagonists, prazosin 및 corynanthine에 의하여는 영향을 받지 아니하였다.
4. *d*-Amphetamine 투여시 부동자세 시간은 용량에 비례하여 단축되었고, 이러한 단축효과는 yohimbine에 의하여는 길항되었으나 prazosin에 의하여는 영향을 받지 아니하였다.
5. α-methyl-*p*-tyrosine 이나 reserpine 또는 두 약물을 동시에 전처치 하였을때 clonidine에 의한 부동자세 시간의 단축은 영향을 받지 아니하였다.
6. Desipramine 및 imipramine 같은 항우울제를 장기처치 또는 장기간 전기충격 요법을 가한 새앙쥐에서도 clonidine의 효과는 영향을 받지 아니하였다.

이상의 결과로 보아 새앙쥐의 강제수영 실험에서의 부동자세 시간의 변동은 중추내 noradrenergic neuron의 postsynaptic α_2-adrenoceptor와 밀접한 관련이 있다고 시사되며 이러한 α_2-agonists에 의하여 항진되는 escape-directed behavior는 자기보호를 위한 일종의 충동의 유발로 인한 행동으로 사료된다.