Korean J. of Pharmacology
Vol.22, No.1, pp. 45-50, 1986

# Possible Relationship between Hyperactivity of Central $\alpha_2$-Adrenoceptors and Muricidal Behavior in Olfactory Bulbectomized Rats

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## ABSTRACT

The involvement of central $\alpha_2$-adrenoceptors in the development of muricide (mouse-killing behavior) in the olfactory bulbectomized (OB) rats was strudied. The fractional turnover rate of noradrenaline (NA) in forebrain from OB muricidal rats was significantly lowered than that from sham rats. However, after yohimbine or idazoxan treatment, NA turnover was significantly increased in concert with inhibition of the development of muricide in OB rats. By radioligand binding studies with [$^3$H] yohimbine, the maximum binding ($B_{max}$) of [$^3$H] yohimbine to cerebral cortical membrane from OB rats was significantly higher than that from sham rats without change of affinity for agonists and antagonists ($K_i$).

With these results it was suggested that muricidal behavior of OB rats has a close relevance to the hyperactivity of central $\alpha_2$-adrenoceptor system.

**Key Words:** muricide, olfactory bulbectomized rat, [$^3$H] yohimbine, central $\alpha_2$-adrenoceptor

## INTRODUCTION

The mouse-killing behavior (muricide) of rat has been used as an animal model of depressive illness, since the muricidal behavior induced by olfactor bulbectomy (Bandler and Chi, 1972; Bernstein and Moyer, 1970; Cain, 1974) or by raphe nuclei lesions (Yamamoto and Ueki, 1977; 1978) was selectively inhibited by the administration of antidepressants (Horovitz *et al.*, 1965; Ueki *et al.*, 1972). Under the postulation that the underlying mechanism of affective disorders functions, it was suggested that the occurrence of muricide in OB rats was considered to be related with the alteration in central noradrenergic neuron (Bandler, 1971; Moyer, 1968). Nevertheless, the role of central $\alpha_2$-adrenoceptors remained still unclear. Recently, Lee and Hong (1985) demonstrated that $\alpha_2$-adrenoceptor antagonists, yohimbine, rauwolscine and idazoxan delayed the latency of muricidal activity, and strongly prevented the muricidal behavior.

Thus, in the present study, it was undertaken to elucidate the role of central $\alpha_2$-adrenoceptors by determining the alterations in the NA turnover rate and the specific binding of [$^3$H] yohimbine to cerebral cortical membrane. Results were discussed with reference to the role of $\alpha_2$-adrenoceptor in central noradrenergic neurons.

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## MATERIALS AND METHODS

Drugs used in this study were yohimbine HCl (Sigma), idazoxan HCl (RX 781094, Reckitt and Colman), α-methyl-p-tyrosine methyl ester (Sigma), phenylephrine HCl (Sigma), guanfacine HCl (Sandoz) and probenecid (Sigma). The radioligand [$^3$H] yohimbine (specific activity; 84.9 Ci/mmol) was purchased from New England Nuclear Corp. and stored at -40 °C before use.

Male Sprague-Dawley rats weighing 180-220g were used in the experiment. The rats were housed in individual cage (15 × 21 × 17 cm) with wire-mesh walls, and were given food and water ad libitum. They were tested two times for the detection of their individual disposition of spontaneous muricide for 15 min and the non-killer rats were used in all experiments.

The animals were anesthetized with secobarbital sodium (35 mg/kg, i.p.) and the olfactory bulbectomy was conducted with the identical procedure as described by Ueki *et al.* (1972). Rats were sacrificed by decapitation and the forebrain was rapidly isolated and stored at -40 °C until use. NA and 3-methoxy-4-hydroxyphenyl-glycol sulfate (MHPG-$SO_4$) were determined according to the method of Ansel and Beeson (1968), and Meek and Neff (1972), respectively. Before determination of the fractional turnover rate (k) and half life ($t_{½}$) of NA, α-methyl-p-tyrosine methyl ester (200 mg/kg, i.p.) was administered and they were calculated as described by Brodie *et al.* (1966).

Both radioligand specific binding and competition studies with [$^3$H] yohimbine on rat cerebral cortical membrane were conducted according to the method described by Doxey *et al.* (1984). Proteins were assayed by the method of Lowry *et al.* (1951).

The results are expressed as the mean ± S.E.M. The statistical significance of difference was determined by Student's t-test and a P value less than 0.05 was taken as statistically significant.

## RESULTS

In the preliminary study, the concentration of NA in the forebrain homogenates from OB rats was

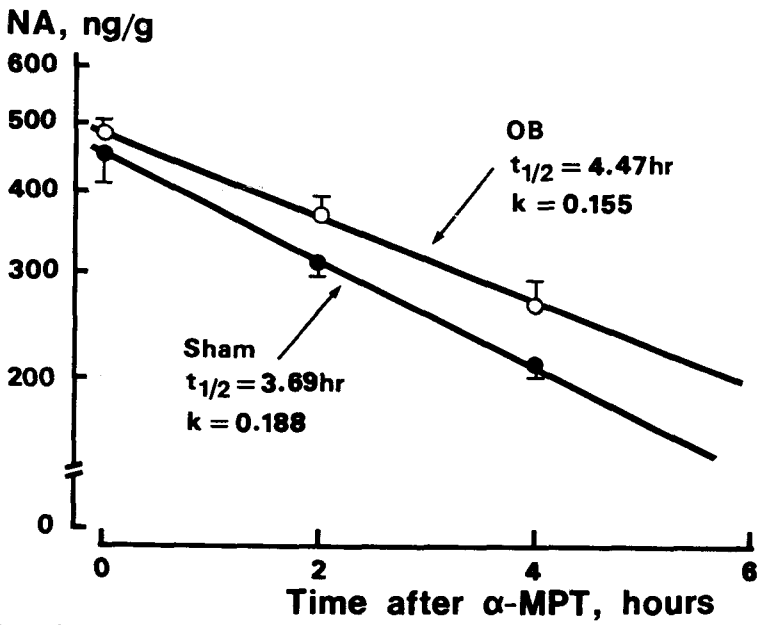


**Fig. 1.** Comparison of noradrenaline (NA) turnover in the forebrain between sham operated and olfactory bulbectomized (OB) rats. NA was measured after pretreatment with α-methyl-p-tyrosine methyl ester (α-MPT; 200 mg/kg, i.p.).

not different from the sham rats. Novertheless, the fractional turnover rate of NA in the forebrain obtained from OB rats was significantly lowered than that from sham rats (OB rats, $t_{1/2}$ =4,47 hr. va. sham, $t_{1/2}$ = 3.69hr) as shown in Fig. 1.

After yohimbine or idazoxan pretreatment the muricidal behavior was abolished. Then, the forebrain was isolated for determination of NA and MHG-$SO_4$ hours after these treatments. The content of NA was diminished in the forebrain and the level of MHPG-$SO_4$ was instead raised by these treatments (Table 1).

**Table 1.** Effect of $\alpha_2$-adrenoceptor antagonists on the turnover rate of noadradenaline (NA) in the olfactory bulbectomized rats

| | n | Na | MHPG-$SO_4$ | MHPG-$SO_4$/NA |
|---|---|---|---|---|
| | | | (ng/g wet tissue weight) | |
| Saline | 6 | 497.32 ± 37.72 | 133.23 ± 7.54 | 0.27 ± 0.02 |
| Yohimbine, 1 mg/kg | 7 | 363.20 ± 25.68[a] | 175.09 ± 14.86[b] | 0.48 ± 0.04[c] |
| Idazoxan, 1 mg/kg | 7 | 274.19 ± 37.49[c] | 156.63 ± 10.60[a] | 0.57 ± 0.04[c] |

n, Numbers of experiments.
[a] $p<0.05$; [b] $p<0.01$; [c] $p<0.001$, Significantly different from the corresponding level of saline group.

**Table 2.** Scatchard analysis of [$^3$H] yohimbine binding to cerebral cortical membranes from sham and olfactory bulbectomized (OB) rats

| Radioligand | Groups | n | $K_d$ (nM) | $B_{max}$ (fmol/mg protein) |
|---|---|---|---|---|
| [$^3$H] yohimbine | Sham rats | 7 | 5.2 ± 1.0 | 23.3 ± 4.0 |
| | OB rats | 7 | 7.8 ± 2.2 | 46.4 ± 7.8[a] |

n, Number of experiments.
$K_d$ values were estimated from the slope of least square regression line.
[a] $p<0.001$, Significantly different from the corresponding level of the sham rats.

**Table 3.** Inhibition of [$^3$H] yohimbine binding to rat cerebral cortical membrane by α-adrenoceptor agonists and antagonists

| Radioligand | Unlabelled drugs | $K_i$ (nM) | |
|---|---|---|---|
| | | Sham rats | OB rats |
| [$^3$H] yohimbine | Phenylephrine | 57.8 ± 9.4 | 41.7 ± 12.1 |
| | Guanfacine | 51.5 ± 13.1 | 39.8 ± 8.4 |
| | Yohimbine | 19.8 ± 5.9 | 28.9 ± 7.4 |
| | Idazoxan | 10.7 ± 0.9 | 10.5 ± 2.1 |

Each value represents the mean ± S.E.M. from 4 to 6 determinations in duplicate.
$K_i$ values were calculated from the equation, $K_i = IC_{50}/(1 + [[^3H]\ \text{yohimbine}]/K_d)$ described by Cheng and Prusoff (1973).
$IC_{50}$ values were determined by log-probit analysis of α-adrenoceptor drugs.

The specific binding of [$^3$H] yohimbine to the cerebral cortical membrane was estimated as shown in Table 2. The maximum binding capacity ($B_{max}$) of [$^3$H] yohimbine to the cerebral cortical membrane was estimated shown in Table 2. The maximum binding capacity ($B_{max}$) of [$^3$H] yohimbine in OB rats was $46.4 \pm 7.8$ fmol/mg protein. It was significantly higher than that of sham group ($p<0.001$) without any difference in $K_d$ values. The competition studies were undertaken using various concentrations of $\alpha$-adrenoceptor drugs, phenylephrine ( $\alpha_1$-agonist), guanfacine ( $\alpha_2$-agonist), yohimbine and idazoxan ( $\alpha_2$-antagonists) on the rat cerebral cortical membrane. The affinity constant ($k_i$ .) for these agonist and antagonists were not distinctly altered by OB (Table 3).

## DISCUSSION

The muricidal behavior itself has been considered as an experimental model in connection with a depressive illness in human beings, since mouse-killing behavior of rats was sensitively inhibited by the antidepressants and electroconvulsive shock (Garzón *et al.*, 1979; Suwandi *et al.*, 1982; Ueki *et al.*, 1972). On the other hand, the mechanism of action of tricyclic antidepressants has focused on the central noradrenergic (Glowinski and Axelrod, 1964; Iversen, 1975; U'Prichard *et al.*, 1978) and serotonergic neurotransmission (Tang and Seeman, 1980). Furthermore, the chronic administration of desipramine was known to be associated with the development of subsensitivity of $\alpha_2$-adrenoceptors (Crews and Smith, 1978; Smith *et al.*, 1981; Sugrue, 1981). In view of these considerations, the muricidal behavior has been widely investigated in relation to the central noradrenergic neuronal function (Oishi and Ueki, 1978; Yamamoto *et al.*, 1982). In an effort to explore the underlying mechanism of the motivation of killing behavior, we have previously reported the inhibitory effect of $\alpha_2$-adrenoceptor antagonists on the latency and incidence of muricide in OB rats (Lee and Hong, 1985).

In the present study, NA turnover rate in the forebrain from OB rats was significantly lowered than that from sham rats, though the NA level from OB rats showed no difference from sham group. On the other hand, after yohimbine or idazoxan treatment the MHPG-$SO_4$ content was significantly increased association with the inhibition of muricide. These results prompt the suggestion that central noradrenergic neuron may be hyperactive in rats with OB muricide.

In addition, the evidence that the numbers of radioligand [$^3$H] yohimbine binding sites were higher in the forebrain from OB muricidal rats strongly support to postulate the hypothesis that the development of muricide in OB rats is closely related with the hyperactivity of central $\alpha_2$-adrenoceptors. However, it is still unidentified whether the lowered turnover rate of NA in the forebrain from OB rats can be ascribed to the increased activity of autoinhibition mechanism as demonstrated in the peripheral sympathetic nerve endings (Langer, 1981; Starke *et al.*, 1975; 1977).

In conclusion, it was suggested that the enhancement of central $\alpha_2$-adrenoceptor system may lead to decrease NA turnover rate, by which mechanism muricidal behavior may be evoked in OB rats.

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=국문초록=

## 후신경구절제 흰쥐에서 Muricide 발생기전으로서 $\alpha_2$-Adrenoceptors의 기능항진에 관한 연구

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이원석, 임병용, 홍기환

후신경구절제(OB) 흰쥐에 있어서 muricide의 발생은 중추성 $\alpha$-adrenoceptors의 기능항진과 밀접한 관계가 있다는 가설하에 다음 실험을 행하였다.

Muricide를 일으키는 OB 흰쥐의 전뇌내 noradrenaline(NA)의 전환율은 대조군에 비하여 현저히 낮았으나 $\alpha_2$-adrenoceptor 길항약물인 yohimbine이나 idazoxan 투여시 NA 전환은 muricide의 억제와 함께 현저히 증가되었다. OB 흰쥐의 전뇌 피질막의 [$^3$H] yohimbine에 대한 최대결합능(Bmax)은 대조군에 비하여 현저히 높았다. $\alpha$-Adrenoceptor 효능약물 및 길항약물에 대한 친화도는 아무런 변동이 없었다.

이상의 결과로 보아 OB 흰쥐에서 야기되는 muricide는 중추성 $\alpha_2$-adrenoceptors 의 기능 항진과 밀접한 관련이 있다고 사료되는 바이다.