The role of α-adrenoceptors in the regulation of pentylenetetrazol convulsions in mice

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The role of the individual monoamines in pentylenetetrazol (PTZ; leptazol) convulsions has been examined with the aim of accounting for increased susceptibility of mice and rats to PTZ convulsions after brain monoamines have been reduced by reserpine (Chen et al 1954; Little & Conrad 1960). Since the susceptibility to PTZ convulsions was increased when noradrenaline (NA) content declined after treatment with α-methyltyrosine, disulfiram, FLA-63 or 6-hydroxydopamine (6-OHDA) (Corcoran et al 1973; Kilian & Frey 1973; Oishi et al 1979), NA is thought to be involved in the regulation of PTZ convulsions although 5-hydroxytryptamine (5-HT) also contributes to the regulation of convulsions (Lessin & Parkes 1959; Diaz 1974). Kilian & Frey (1973) found that the threshold for the PTZ convulsions was lowered by propranolol, and suggested that there is a participation of β-adrenoceptors in the modulation of PTZ convulsions. In the present study, we have attempted to clarify a participation of α-adrenoceptors in regulating susceptibility to PTZ convulsions.

Male CF 1 strain mice (Kyushu University Institute of Laboratory Animals), 25–35 g, were briefly restrained while a 3% solution of PTZ was infused into a lateral tail vein at a constant rate of 0·097 ml min^{-1} with a infusion pump (Harverd Apparatus 975). Intravenous infusion of PTZ induced the following sequence of responses; after a few initial twitches, generalized clonic seizures without righting reflex ensued, leading then to myoclonic jerks, and finally tonic extension. In the first experiment, the lengths of PTZ infusion required to elicit the twitch, generalized clonic seizures and tonic extension of hindlegs were recorded, and the effects of phentolamine mesylate (Regitin, Ciba-Geigy), clonidine hydrochloride (Boehlinger) and 6-OHDA hydrobromide (Sigma) on each convulsive threshold were examined. Each threshold was expressed as the mean ± s.e.m. dose of PTZ kg^{-1} weight. 50 μg of 6-OHDA hydrobromide was dissolved in 10 μl of 0·9% NaCl (saline) —0·1% ascorbic acid solution and injected into the lateral ventricle under ether anaesthesia 10 days before PTZ challenge. The other drugs were dissolved in 0·9% NaCl and injected (0·1 ml/10 g) intraperitoneally 30 min before PTZ challenge. We previously reported that 50 μg of 6-OHDA caused reduction in NA and DA contents of mouse whole brain down to 30% and 50% of the control, respectively (Fukuda et al 1975). In the second experiment, the effect of clonidine on the incidence of generalized clonic seizures and tonic extension induced by PTZ (20–60 mg kg^{-1}, i.v.) was examined in order to further clarify its effect on the PTZ convulsive thresholds.

Table 1 shows the effects of drugs on the threshold amount of PTZ needed to elicit each seizure. Phentolamine, 10 mg kg^{-1}, induced a statistically significant elevation of the threshold for twitch ($P < 0·01$), generalized clonic seizures ($P < 0·05$) and tonic extension ($P < 0·01$). In contrast, clonidine 1 mg kg^{-1} induced a statistically significant reduction of the thresholds for twitch ($P < 0·001$) and tonic extension ($P < 0·05$). This facilitatory effect of clonidine, 1 mg kg^{-1}, on the PTZ convulsion was almost antagonized by the simultaneous treatment with phentolamine 5 mg kg^{-1}. 6-OHDA 50 μg, applied intraventricularly, induced significant reduction of the threshold for tonic extension ($P < 0·05$). In 6-OHDA-treated mice, phentolamine, 10 mg kg^{-1}, caused no significant change in each threshold for PTZ convulsions, although clonidine, 1 mg kg^{-1}, significantly reduced the thresholds for twitch ($P < 0·05$) and generalized clonic seizures ($P < 0·05$).

As shown in Table 2, clonidine, 1 mg kg^{-1}, significantly enhanced both incidences of the generalized clonic seizures and tonic extension induced by intravenous injection of a fixed dose of PTZ.

Table 1. Effects of drugs on pentylenetetrazol (PTZ) seizure threshold in mice. 6-OHDA was injected intraventricularly 10 days before PTZ challenge. The other drugs were administered intraperitoneally 30 min before PTZ challenge. Each value is the mean ± s.e.m. of 8 or 9 animals.

	PTZ seizure threshold (mg kg^{-1})		
Treatment	Twitch	Generalized clonic seizures	Tonic extension
Saline, 0·1 ml/10g	53·9 ± 2·0	66·9 ± 4·2	135·0 ± 7·8
Phentolamine, 5 mg kg^{-1}	59·3 ± 2·6	71·4 ± 3·1	146·1 ± 6·5
10 mg kg^{-1}	66·6 ± 3·3**	79·6 ± 4·2*	171·5 ± 6·6**
Clonidine, 0·5 mg kg^{-1}	45·1 ± 1·9**	62·7 ± 2·2	124·6 ± 8·6
1 mg kg^{-1}	42·9 ± 1·5***	60·9 ± 2·0	107·1 ± 8·8*
Clonidine, 1 mg kg^{-1} + phentolamine, 5 mg kg^{-1}	47·9 ± 3·0	63·6 ± 3·2	142·7 ± 6·5
6-OHDA, 50 μg	50·1 ± 2·1	61·5 ± 2·9	112·1 ± 7·2*
6-OHDA, 50 μg + phentolamine, 10 mg kg^{-1}	53·8 ± 1·8	65·4 ± 2·4	117·3 ± 8·3
6-OHDA, 50 μg + clonidine, 1 mg kg^{-1}	41·4 ± 2·3†	52·5 ± 2·5†	92·9 ± 6·7

* $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$, compared with the saline treated group; † $P < 0·05$, compared with the 6-OHDA treated group (two-tailed Student's *t*-test).

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Table 2. Effect of clonidine (1 mg kg^{-1} i.p., 30 min before) on the incidence of generalized clonic seizures and tonic extension induced by intravenous injection of fixed dose of PTZ.

PTZ (mg kg^{-1})	Generalized clonic seizures		PTZ (mg kg^{-1})	Tonic extension	
	Saline	Clonidine		Saline	Clonidine
20	0/10	3/10	40	0/10	4/10*
25	4/10	9/10*	45	3/10	7/10
30	9/10	—	50	4/10	7/10
			55	5/10	9/10
			60	9/10	—

Each value is the incidence of each convulsion.
* $P < 0.05$, compared with the saline treated group (Fisher's exact probability test).

The present study revealed that susceptibility to PTZ convulsions was decreased by phentolamine and increased by clonidine, indicating the existence of α-adrenoceptors that facilitate the development of PTZ convulsions.

Intraventricularly applied 6-OHDA increased the susceptibility of mice to PTZ convulsions in agreement with the findings in rats (Corcoran et al 1973; Oishi et al 1979). Phentolamine showed an effect on the PTZ convulsions opposite from that of 6-OHDA. It has been reported that the mechanism mediated by presynaptic α-adrenoceptors modulates transmitter release from central noradrenergic neurons (Farnebo & Hamberger 1971; Starke & Montel 1973), and some effects of clonidine may be mediated by presynaptic α-adrenoceptors (Paalzow & Paalzow 1976; Strömbom 1976). In addition, it has been also reported that electrical activity of NA neurons of the locus coeruleus shows inhibition by adrenaline and activation by α-antagonists (Cedarbaum & Aghajanian 1976). In this study, the effect of phentolamine on PTZ seizure thresholds was attenuated in 6-OHDA-treated mice. These results, taken together, suggest the existence of α-adrenoceptors which inhibit the function of the presynaptic NA neuron and increase susceptibility to PTZ convulsions. However, the effect of clonidine on the PTZ seizure threshold was not attenuated by 6-OHDA. This may be due to the increase in turnover and of transmitter supersensitivity in the remaining noradrenergic neurons (Kalisker et al 1973; Spon et al 1976). Low dosage of clonidine is thought to be selective to the presynaptic α-adrenoceptors (Strömbom 1976) but we could not find any change in the PTZ threshold with lower doses of clonidine (0·05–0·2 mg kg^{-1} i.p.).

The great reduction by clonidine of the threshold for twitch may be partially due to its own hypotensive effect, which quickens initial PTZ influx. However, we confirmed the facilitatory effect of clonidine on PTZ convulsions in the second experiment. In addition, it was reported that intraperitoneal injection of clonidine, 0·5 mg kg^{-1} caused an almost similar degree of hypotensive effect to that of clonidine 1mg kg^{-1} (Razzak et al 1977), whereas in this experiment clonidine decreased PTZ seizure threshold in a dose-dependent manner.

In conclusion, these results suggest that susceptibility to PTZ convulsions is regulated by facilitatory α-adrenoceptors.

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