CARDIOVASCULAR CHANGES INDUCED BY LARGE DOSES OF CLONIDINE IN MICE*

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Abstract—Clonidine at doses of 0.5–1.0 mg/kg i.p. produced an initial rise followed by a sustained fall in blood pressure. The initial pressor response became more marked and the onset of hypotensive effect was delayed as the dose was increased to large doses such as 10–50 mg/kg given intraperitoneally. The heart rate was markedly reduced soon after clonidine administration and the bradycardia lasted for more than 2 hours. Both the initial pressor and subsequent hypotensive effects of clonidine were reduced by pretreatment with phentolamine, the initial pressor effects were suppressed by propranolol which did not affect the hypotensive effects. This initial pressor effect was potentiated while the hypotensive effect was reduced after bilateral vagotomy and pretreatment with either 6-hydroxydopamine or atropine. The bradycardia was significantly reduced by propranolol, atropine and bilateral vagotomy. Central sympathetic as well as parasympathetic mechanisms may be involved in cardiovascular changes after large doses of clonidine in urethanized mice.

Clonidine is a potent antihypertensive drug which produces a marked blood pressure fall and bradycardia when administered in microgram doses (1, 2). It has been suggested that the hypotensive effect of clonidine is a result of the reduction of the central sympathetic tone as hypotension occurs when the drug is injected into either the cisterna magna or the lateral venticle in several animal species (3, 4, 5), and that the site of central action of clonidine is located at the level of the medulla (6, 7, 8). Furthermore, it has been reported that clonidine has a direct stimulant action on the adrenergic α-receptor in peripheral smooth muscle (9, 10, 11), and that the hypotensive effect is abolished by pretreatment with adrenergic α-receptor blocking agents (12). It has been proposed that the central hypotensive action of clonidine is the result of stimulation of the central noradrenaline receptor (13, 14, 15).

On the other hand, clonidine given intraperitoneally in large doses induces intense tremor and aggressive behavior although it causes sedation at low doses in mice (16). The authors have also confirmed the same aggressive behavior as induced by clonidine and found that large doses of the drug induce automutilation in mice (17). The biting behavior induced by clonidine was found to be related to central noradrenergic as well as cholinergic mechanisms (18).

To our knowledge there is no documentation concerning the cardiovascular changes...
induced by clonidine in mice especially when large doses are given. Effects of low doses of the drug have been extensively studied in many animal species except mice.

We therefore carried out experiments to determine the cardiovascular changes induced by large doses of clonidine and the influences of drugs related to adrenergic and cholinergic mechanisms.

Mice were used in the experiment in order to elucidate the neural mechanisms of the cardiovascular effect, in correlation with such a mechanism of aggressive behavior induced by large doses of clonidine.

MATERIALS AND METHODS

Male ddN strain mice weighing 20–25 g were anesthetized with urethane 1.6 g/kg i.p. Carotid arterial pressure was measured by means of a pressure transducer (Toyo Sokki, LPU-0.5-260-0-III) connected with biophysiograph (Sanei Sokki, SYS-180). The heart rate was measured with a cardiocapnometer (Nihon Koden, RT-5) triggered by the electrocardiogram lead-II, and recorded simultaneously with the carotid pressure on a rectigraph (Sanei Sokki, 8S). Drugs used in the experiment were clonidine hydrochloride (St 155, Catapres; C. H. Boehringer Sohn), phentolamine mesylate (Regitine; Ciba-Geigy), propranolol hydrochloride (Inderal; Sumitomo), atropine sulphate (Iwaki) and 6-hydroxydopamine hydrobromide (6-OHDA: Sigma). All drugs were dissolved or diluted in a 0.9% saline solution and administered i.p., except 6-OHDA which was dissolved in a 0.9% saline solution containing ascorbic acid at a concentration of 0.1% and administered at a volume of 10 μl/animal intraventricularly (i. vent).

The results were statistically analyzed using Student's t-test.

RESULTS

Effects of clonidine on blood pressure and heart rate in mice

In urethanized mice, the blood pressure was about 100–105 mmHg and the heart rate about 700–800 beats/min. When clonidine was given to mice i.p. at doses of 0.5–1.0 mg/kg, carotid blood pressure was gradually lowered by 20–30 mmHg, after a temporary rise of 5–15 mmHg. The heart rate was markedly reduced by 40–400 beats/min. A representative result is shown in Fig. 1. The effects of clonidine lasted for more than 2 hours, although the heart rate recovered faster than did the rate of blood pressure. With large doses such as 10–50 mg/kg i.p., the initial rise of blood pressure was more marked (25–30 mmHg), and was followed by a temporary fall and secondary rise in blood pressure particularly at a dose of 50 mg/kg i.p.. The onset of sustained hypotension was more delayed and that of bradycardia was more rapid as the dose of clonidine was increased (Figs. 1, 2).

The influence of various drugs and vagotomy on the cardiovascular change induced by a fixed dose of 50 mg/kg i.p. of clonidine was also investigated.

Effect of phentolamine

Phentolamine 10 mg/kg i.p. caused a slight fall in blood pressure and slight decrease in
heart rate, though both were insignificant. Phentolamine administered i.p. at a dose of 10 mg/kg 10 min before clonidine, significantly reduced both the initial rise and later sustained fall in blood pressure induced by clonidine, and increased the secondary blood pressure rise but produced no significant change in the bradycardia (Fig. 3).
Effect of phentolamine on the cardiovascular changes induced by clonidine 50 mg/kg i.p. in urethanized mice. Upper panel shows mean changes in arterial pressure. Lower panel shows mean changes in heart rate (n = 6). ○ ○ ○ : phentolamine 10 mg/kg i.p., ■ ■ ■ : clonidine 50 mg/kg i.p. as control, ○ ○ ○ : clonidine 50 mg/kg i.p. 10 min after phentolamine 10 mg/kg i.p. In this and subsequent figures each point is a mean with vertical bars indicating the standard error. *p < 0.05, **p < 0.01, ***p < 0.001 significantly different from the control. Arrow A indicates phentolamine administration, and B clonidine administration.

Effect of propranolol

Propranolol at a dose of 10 mg/kg i.p. produced a slight, temporary rise in blood pressure and decreased significantly the heart rate by 100-150 beats/min. When clonidine was administered i.p. 10 min after propranolol in a dose of 10 mg/kg i.p., the initial sharp pressor response and the secondary blood pressure rise was significantly reduced, but the sustained hypotensive effect was the same as the control. The bradycardia induced by clonidine was significantly reduced by propranolol (Fig. 4).

Effect of 6-OHDA

6-OHDA caused no significant changes in blood pressure or heart rate of mice. The initial pressor response and bradycardia induced by clonidine remained unchanged but the sustained hypotensive effect was significantly reduced after pretreatment with 6-OHDA (50 µg/animal, i. vent.) 7-10 days before clonidine administration (Fig. 5).

Effect of atropine and bilateral vagotomy

Although atropine (5 mg/kg i.p.) caused no significant change in either blood pressure or heart rate, the initial pressor response induced by clonidine was potentiated and prolonged, whereas the sustained hypotensive effect and bradycardia were markedly reduced by atropine administered 10 min before clonidine (Figs. 6, 7). Bilateral vagotomy had almost the same influence as atropine on the effect of clonidine (Fig. 7).
Effect of propranolol on the cardiovascular changes induced by clonidine 50 mg/kg i.p. in urethanized mice. Upper panel shows mean changes in arterial pressure, and lower panel shows mean changes in heart rate (n=6). •—•: propranolol 10 mg/kg i.p., ■—■: clonidine 50 mg/kg i.p. as control, □—□: clonidine 50 mg/kg i.p. 10 min after propranolol 10 mg/kg i.p. *p<0.05, **p<0.01, ***p<0.001 significantly different from the control. Arrow A shows propranolol administration and B clonidine administration.

Effect of 6-OHDA on the cardiovascular changes induced by clonidine 50 mg/kg i.p. in urethanized mice. Upper panel shows mean changes in arterial pressure and lower panel mean changes in heart rate (n=6). ○—○: clonidine 50 mg/kg i.p. as control, ●—●: clonidine 50 mg/kg i.p. 7-10 days after 6-OHDA 50 μg/animal i.vent. *p<0.05. **p<0.01 significantly different from the control.
FIG. 6. Effect of atropine on the cardiovascular changes induced by clonidine 50 mg/kg i.p. in urethanized mice. Upper panel shows mean changes in arterial pressure and lower panel mean changes in heart rate (n=6). ---: clonidine 50 mg/kg i.p. as control, •: clonidine 50 mg/kg i.p. 10 min after atropine 5 mg/kg i.p. *p < 0.05, **p < 0.01, ***p < 0.001 significantly different from the control.

FIG. 7. Representative records of the effects of atropine and bilateral vagotomy on the cardiovascular changes induced by clonidine 50 mg/kg i.p. in urethanized mice. A: the effect of atropine 5 mg/kg i.p. B: the effect of bilateral vagotomy.

DISCUSSION

Clonidine at large doses produced a sharp temporary rise followed by a marked sustained fall in blood pressure and a marked bradycardia in urethanized mice. This cardiovascular
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change induced by clonidine was qualitatively similar to that reported in other animal species such as rats, cats and rabbits in either conscious or anesthetized states (19, 20, 21, 22). However, when clonidine was given in the large dose of 50 mg/kg i.p., the initial pressor response was followed by a temporary fall and secondary rise in blood pressure which lasted for about 10 min prior to sustained hypotension.

The initial pressor response to large doses of clonidine appears to be due to a direct stimulation of adrenergic α-receptors of peripheral blood vessels, since the response was not altered after ablation of central catecholaminergic neurons induced by intraventricular injection of 6-OHDA and was reduced by systemic administration of phentolamine. Propranolol also partially reduced the initial pressor response to clonidine. Such is attributed to a nonspecific membrane stabilizing action as seen with local anesthetics (23, 24, 25). Both atropine and bilateral vagotomy potentiated the pressor response to clonidine and the relative dominance of the sympathetic tone and a lack of reduction in cardiac output would explain this potentiation. The sharp fall in blood pressure following the initial pressor response observed with a dose of 50 mg/kg i.p. may be due to a baroreceptor reflex, while the secondary rise in blood pressure may be the result of a direct action of clonidine on the peripheral vascular muscle.

The sustained hypotensive effect of clonidine was significantly reduced by phentolamine but was not influenced by propranolol. As phentolamine is known to penetrate the blood-brain barrier (26), this result would support the hypothesis proposed by Schmitt et al. (19) that clonidine stimulates central adrenergic α-receptors. The hypotensive effect of clonidine was also reduced by intraventricular injection of 6-OHDA which is known to produce widespread destruction of central noradrenergic neurons (27, 28). The present results in mice are in good agreement with those of Dolley and Reid (20) in conscious as well as in anesthetized rabbits but do not support the data of Haeusler and Finch (29) and Finch et al. (11) who reported that 6-OHDA did not significantly modify the clonidine-induced hypotension in conscious and anesthetized rats. It would thus appear that the central noradrenergic neurons are also required to elicit the hypotensive effect of clonidine and that clonidine acts not only on the postsynaptic receptors but also in the same way on this integrity of noradrenergic neurons in the central nervous system. The hypotensive effect of clonidine in mice was also abolished by either atropine or bilateral vagotomy. This result indicates that the hypotensive effect is dependent on intact vagal activity, and is in good agreement with the finding of Zaimis (30) in rats. The hypotensive effect of clonidine was not dose-dependent at the dose levels used in the present experiments. It has been reported, however, that clonidine induces a dose-dependent fall in blood pressure when much lower doses are used in rats (11) and cats (31). The doses of clonidine we used here may be too large to cause the dose-dependent effect, i.e. the drug induced a maximum hypotension even at a dose of 0.5 mg/kg i.p.

Clonidine caused marked bradycardia even during the period of initial pressor response. The bradycardia would obviously be caused by an increase in vagal tone and a decrease in sympathetic tone resulting from the central action of clonidine.
The present data suggest that central sympathetic as well as parasympathetic mechanisms are involved in the cardiovascular changes which occur when large doses of clonidine are given to mice. It is also assumed that the central adrenergic mechanism plays a major role in inducing aggressive behavior (18).

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