Modulation of Cardiovascular Depressant Action of Clonidine by Pentobarbital Anesthesia in Rats

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IMAI, Y., ABE, K., SASAKI, S., MINAMI, N., NIHEI, M., MUNAKATA, M., SEKINO, H. and YOSHINAGA, K. Modulation of Cardiovascular Depressant Action of Clonidine by Pentobarbital Anesthesia in Rats. Tohoku J. Exp. Med., 1989, 157 (3), 221–227 —— The influence of pentobarbital anesthesia (5 mg/100 g, i.p.) on the cardiovascular depressor effect of clonidine administered intracerebroventricularly (i.c.v.) and intravenously (i.v.) was examined in rats. In conscious rats the hypotensive potency of i.v. clonidine [effective dose of clonidine which induced a decrease in blood pressure of 15 mmHg; ED_{15} = 7 (µg/kg)] is greater than that of i.c.v. clonidine (ED_{15} = 24.3), while in anesthetized rats that of i.c.v. clonidine (ED_{15} = 0.7 µg/kg) was greater than that of i.v. clonidine (ED_{15} = 3.5 µg/kg). Pentobarbital anesthesia potentiated the hypotensive potency of i.c.v. clonidine in conscious rats by 50 times, while it potentiated that of i.v. clonidine only by 2 times. The bradycardic potency of i.v. and i.c.v. clonidine was also potentiated by pentobarbital anesthesia. These pieces of evidence suggest that in conscious rat, the hypotensive action of i.c.v. clonidine is opposed by a centrally mediated hypertensive effect of the drug and that pentobarbital anesthesia suppresses the clonidine sensitive pressor center and so potentiates the hypotensive effect of i.c.v. clonidine ——— blood pressure; cerebroventricular injection; clonidine; heart rate; pentobarbital

There is a volume of evidence to indicate that the hypotensive and bradycardic effects of clonidine are mediated by α2-adrenoceptor and/or imidazole receptor mechanisms in the depressor site of the medullary vasomotor center (Schmitt 1977; Kobinger 1978; Ernsberger et al. 1987). Studies which have shown that centrally administered clonidine caused greater hypotensive effects than the same dose given intravenously (Kobinger 1978) have been a part of that evidence which indicates a central mode of action. Such results have been obtained in several species of animal mainly under anesthetized conditions. However, there is no report of a significant difference in the hypotensive potency of clonidine via different administration route in rats, the most popular experimental animal. We

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recently reported that in conscious rats the hypotensive effect of centrally administered clonidine was very weak and less potent than that of peripherally administered clonidine (Imai et al. 1986). Taken together this information suggests that anesthesia affects the cardiovascular effect of clonidine, and indeed it has been reported that pentobarbital anesthesia potentiated the hypotensive effect of i.v. clonidine in rats (Trolin 1975). In the present study we have studied the cardiohemodynamic effects of i.v. and i.c.v. clonidine in conscious rats and compared these with the responses seen in pentobarbital anesthetized rats.

**Methods**

Long-Evans rats weighing 300–380 g and aged 25–35 weeks were used. An intracerebroventricular (i.c.v.) cannula was implanted chronically in animals under alphaxalone anesthesia (Alfatesin®, Glaxo, 9 mg/kg). Cannulae were made of PE 20 polyethylene tubing (Clay-Adams) and were implanted with the aid of stereotaxic instruments into the left lateral ventricle at coordinates AP 1.0, L 1.5, H 5.0, using the bregma as reference. Stainless steel anchoring screws and dental acrylic cement served to secure the cannula, which was filled with saline until the time of the experiment. Animals were allowed a week to recover before the left femoral artery and vein were catheterized with polyethylene tubing under ether anesthesia. The tip of the arterial catheter (PE 100) was tapered by heating and PE 50 tubing was used for the venous catheter. The catheters were passed subcutaneously and brought out at the neck. They were filled with heparinized saline (1000 IU/ml) and sealed by heating. Rats were allowed to recover for at least 24 hr after surgery. Blood pressure was recorded from the femoral arterial catheter using a P23lb Statham pressure transducer (Oxford, CA, USA) and pulse period was monitored with a period meter (Baker Institute model Unicon kfh 122B, Prahran, Australia). Both parameters were recorded continuously on a rectilinear recorder (Type BK 12, San-Ei Instrument, Tokyo).

The drugs used in the present study were: Clonidine hydrochloride (Boehringer Ingelheim, Ingelheim, FRG) and pentobarbital sodium (Nembutal®, Abbott Laboratories, North Chicago, IL, USA). Clonidine was dissolved in physiological saline. Vehicle or drug solutions were administered i.c.v. or i.v. volumes of less than 15 µl or 150 µl, respectively.

**Experimental protocol**

*Cardiohemodynamic effects of i.c.v. and i.v. clonidine in conscious rats*

The dose–response relationship for the cardiohemodynamic effect of i.c.v. clonidine \( (n = 9) \) and i.v. clonidine \( (n = 6) \) was examined in conscious rats. As a preliminary experiment, the cardiohemodynamic effect of i.c.v. clonidine in doses of 0.1, 0.3, and 1 µg/kg was examined. The dose–response curves for i.c.v. and i.v. clonidine were constructed with doses of 3, 10 and 30 µg/kg. Two hr were allowed for recovery following the 3 µg/kg dose, and at least 24 hr following 10 µg/kg.

*Cardiohemodynamic effects of i.v. and i.c.v. clonidine in anesthetized rats*

Pentobarbital in a dose of 5 mg/100 g was administered intraperitoneally to 5 rats in which arterial, venous and cerebroventricular cannulae had been implanted beforehand. The dose–response relationship for i.c.v. clonidine in doses of 0.3, 1 and 3 µg/kg and i.v. clonidine in doses of 0.3, 1, 3 and 10 µg/kg was examined in each group of 5 rats. At least 2 hr were allowed for recovery following the doses and 24 hr before the highest dose via each administration route. Pentobarbital (1–2 mg/100 g) was added during the course of the experiment to maintain adequate levels of anesthesia. The rats were re-anesthetized before the highest dose.
**Statistical method**

All values reported are the mean ± s.e. Dose–response curves were compared by analysis of covariance. The effective dose of clonidine which induced decreases in mean arterial pressure of 15 mmHg (ED$_{15}$, μg/kg) was estimated from the linear regression equation of the dose-response curve.

**RESULTS**

**Cardiohemodynamic effects of i.c.v. and i.v. clonidine in conscious rats**

The hypotensive and bradycardic effects of i.c.v. clonidine in doses of 0.1 and 0.3 μg/kg were minimal and measurable changes were only obtained with doses above 3 μg/kg. I.c.v. clonidine induced an initial transient increase in blood pressure which was followed by prolonged hypotension (Fig. 1, upper and middle panels). The response characteristics of pulse period to i.c.v. clonidine were different at each dose level, although both bradycardia and tachycardia were always observed. Fig. 1 (bottom panel) shows a typical response of pulse period to a 30 μg/kg dose of i.c.v. clonidine. At this level an increase in pulse period (bradycardia) was followed by prominent decrease (tachycardia). The hypotensive response occurred despite the marked decrease in pulse period (tachycardia). I.v. administration of clonidine caused an initial steep increase in arterial pressure which was followed by prolonged hypotension. A sharp rise in pulse period (bradycardia) was observed just after i.v. injection but tended to be very transient. Thereafter, the pulse period gradually increased and this bradycardia persisted for some time. The maximum changes in blood pressure and pulse period for each dose of clonidine were measured and dose–response curves were constructed. In the case of i.v. clonidine, the second phase of the pulse period response, i.e. bradycardia, was used for the dose–response curve. As shown in Fig. 2, i.c.v. and i.v. clonidine induced dose–dependent pressor responses,

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**Fig. 1.** A typical trace of the cardiovascular effect of clonidine administered intracerebroventricularly (i.c.v.) to conscious rats. AP, pulsatile arterial pressure; MAP, mean arterial pressure; PP, pulse period.
hypotension, and increases in pulse period (bradycardia). I.c.v. clonidine also induced a dose-dependent decrease in pulse period (tachycardia), while i.v. clonidine did not produce tachycardia at any dose. The dose-response curve for the hypotensive effect of i.c.v. clonidine was located to the right of that for i.v. clonidine in conscious rats \[ F(1, 41) = 5.3, p < 0.05 \]. The ED\text{15} values of i.c.v. and i.v. clonidine were 24.3 and 7.0, respectively. With regard to its bradycardic action the potency of i.v. clonidine was not significantly different from that of i.c.v. clonidine.

**Cardiohemodynamic effects of i.c.v. and i.v. clonidine in anesthetized rats**

Pentobarbital anesthesia caused gradual decreases in blood pressure and pulse period (tachycardia); effects which had stabilized within 30 min of i.p. injection of the anesthetic. At this time the mean arterial pressure and pulse period (103 ± 2 mmHg and 153 ± 4 msec) were significantly lower than during the control period [117 ± 2 mmHg (p < 0.01) and 170 ± 5 msec (p < 0.05)]. As shown in Fig. 2, the dose–response curve for the hypotensive effect of i.c.v. clonidine was located to the left of that for i.v. clonidine in anesthetized rats \[ F(1, 31) = 40.2, p < 0.01 \]. The ED\text{15} values for i.c.v. and i.v. clonidine were 0.7 and 3.5, respectively. The dose–response curve for the increase in pulse period (bradycardia) induced by i.c.v.
Anesthesia and Effect of Clonidine

Clonidine was not significantly different from that by i.v. clonidine. A dose-dependent decrease in pulse period (tachycardia) was also observed by i.c.v. clonidine in anesthetized rats.

Comparison of the cardiohemodynamic effects of clonidine in conscious and anesthetized rats

As shown in Fig. 2, the dose-response curve for the hypotensive effect of i.c.v. clonidine in anesthetized rats was located to the left of that for conscious rats [F(1, 38) = 38.4, p < 0.01]. The ED15 values for i.c.v. and i.v. clonidine in anesthetized rats were 0.7 and 3.5, respectively. The dose-response curve in anesthetized rats was significantly steeper than that in conscious rats (p < 0.01). The ED15 for i.c.v. clonidine in conscious rats was 50 times higher than that in anesthetized rats, whereas ED15 of i.v. clonidine in conscious rat was only 2 times higher than that in anesthetized rats. The dose-response curves for increases in pulse period (bradycardia) by i.v. and i.c.v. clonidine in conscious rats were the right and parallel to those seen during anesthesia [i.v.: F(1, 34) = 10.7, p < 0.01 and i.c.v.: F(1, 39) = 40.9, p < 0.01].

DISCUSSION

The present study showed that the depressor and bradycardic effects of clonidine administered i.c.v. were greater than those of like dose administered intravenously (i.v.) to anesthetized rats. This accords with the findings of earlier workers in several other species of anesthetized animals (Schmitt 1977; Kobinger 1978). It is, however, different to the results we have obtained in conscious rats, for which the hypotensive potency of i.c.v. clonidine was less than that of i.v. clonidine. Although the mechanism by which the cardiovascular depressor effect of i.c.v. clonidine was suppressed in conscious rats has not yet been elucidated, it is clear that pentobarbital modulates the centrally mediated cardiovascular depressor action of clonidine.

It is well established that the cardiovascular depressor effects of clonidine are mediated by a central α2-adrenoceptor (Kobinger 1978) and/or imidazole receptor (Ernsberger et al. 1987). Several authors have suggested a centrally mediated pressor action of clonidine (Bousquet and Guertzenstein 1973; Trolin 1975; Kawasaki and Takasaki 1986). We recently observed in conscious rat that the hypotension induced by i.c.v. clonidine was converted to a hypertensive response after pretreatment with i.c.v. yohimbine. The hypotension induced by i.v. clonidine, on the other hand, was only reduced in magnitude by i.c.v. yohimbine, and this might suggest the existence of a centrally mediated hypertensive action of clonidine (Imai et al. 1986). The existence of a suprabulbar pressor center involving by alpha-adrenergic mechanisms has been suggested by several authors (Przuntek et al. 1971; Bousquet and Guertzenstein 1973; Korner et al. 1981). Trolin (1975) reported that the hypotensive effect of i.v. clonidine in conscious rat
was potentiated by pentobarbital anesthesia and decerebration, and concluded
that a pressor effect of clonidine, mediated by a suprabulbar pressor center was
easily suppressed by pentobarbital anesthesia. Kawasaki and Takasaki (1986)
also observed that i.c.v. clonidine produced a dose-dependent and long-lasting
pressor response, but a long-lasting depressor response in pentobarbital anesthe-
tized rats. Taken together, this evidence suggests that in conscious rat, the
hypotensive action of i.c.v. clonidine is opposed by a hypertensive effect of the
drug and that pentobarbital anesthesia suppresses the clonidine-sensitive pressor
center and so potentiates the hypotensive effect of i.c.v. clonidine. The existence
of a pressor component to the centrally mediated response to clonidine in con-
scious rats may explain the small hypotensive effect observed under these condi-
tions. Korner et al. (1981) have also studied the cardiovascular depressor effect
of centrally and intravenously administered clonidine in conscious rabbits.
These authors found that clonidine was 30 to 40 times more potent via the i.e.v.
route. These, combined with the results of the present study, indicate that there
are marked species differences in the nature and mechanisms of the cardiovascular
response to clonidine.

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