Role of Brain Catecholamine in Baroreceptor Reflex in Rabbits

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CAROTID sinus nerves terminate in the nucleus tractus solitarii and the adjacent medial-dorsal regions of the medulla, which are richly innervated with noradrenergic neurons. The section of carotid sinus nerves or destruction of the nucleus tractus solitarii is known to cause a sustained rise in arterial pressure.1,2 It was also reported that a significant change in central catecholamine metabolism was found in hypertensive animals of varied origins.3–5

The present study aims to confirm whether or not carotid sinus baroreceptor reflex could be influenced by depletion of catecholamine in the brain following intraventricular injection of 6-hydroxydopamine (6-OH-DA).

METHODS

Adult rabbits of either sex weighing 2.3 to 3.8 kg were anesthetized with 30 mg/kg of α-chloralose and 750 mg/kg of urethane (half intravenously, half intraperitoneally). They were immobilized with 1 mg/kg of decamethonium and artificially ventilated. The right carotid sinus nerve (CSN) was carefully exposed and laid on a bipolar stainless-steel electrode for electrical stimulation. The left common carotid artery was also exposed for occlusion study. Arterial blood pressure was measured from a femoral artery through a strain-gauge transducer and heart rate was monitored through a heart rate meter triggered by R-R interval of ECG. Pulsatile arterial pressure, mean pressure and heart rate were recorded simultaneously with a multichannel pen recorder.

For administration of 6-OH-DA and other drugs, a cannula was inserted stereotaxically into the right cerebral lateral ventricle of an animal under pentobarbita1 sodium anesthesia 24 h prior to the experiment. In the short term study, the ventricular cannula was placed at the beginning of experiment. 6-OH-DA was dissolved in isotonic saline containing 0.1 % ascorbic acid.

Electrical stimulation of CSN was performed before and 0.5, 1, 2, 3, 4 h after intraventricular injection of drugs. The stimulus parameters were 2–6 V, 10–160 Hz, 0.1 msec pulse width and a duration of 20 sec. The optimal amplitude was adjusted at the beginning of experiment to cause a slight fall in mean arterial pressure at 10 Hz, and then the amplitude was kept constant throughout the experiment for each animal. In most of rabbits, the optimal amplitude was 6 V.

For determination of brain norepinephrine, rabbits were decapitated at the end of the study and brain was dissected into three parts: pons-medulla, hypothalamus, and cortex. Norepinephrine was assayed fluorimetrically with the method of Shellenberger and Gordon.6

RESULTS

I. Short term effects of 6-OH-DA

1. Blood pressure and heart rate. Following intraventricular injection of 6-OH-DA, a transient reduction in blood pressure and heart rate was observed.

The average of mean arterial pressures of 13 rabbits was 93 ± 2.3 mmHg (mean ± S.E.) and that of heart rate 252 ± 9.0 beats/min. When 6-OH-DA in the dose of 500 µg/kg was injected

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Key Words:
Baroreceptor reflex
Carotid sinus nerve stimulation
Blood pressure
Brain norepinephrine
6-Hydroxydopamine
Phentolamine

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This paper was presented at the IV Conference on the Pathogenesis of Hypertension, October 13, 1974, Tokyo.
Fig. 1. Short term effects of 6-OH-DA on the responses of blood pressure and heart rate to CSN stimulation (2-6V, 0.1msec, 20Hz, 20sec)
Ordinate: Responses in blood pressure and heart rate expressed in per cent of those before the injection of 500 μg/kg of 6-OH-DA
Abscissa: Time in hour after the injection. The vertical lines represent S.E.M.

Hypotension response in 6-OH-DA treated group (n=9) was statistically significant at 1 and 2h compared with pre-injection value (p < 0.0025) and bradycardia response (n=8) was also significant up to 3h (p < 0.025). In vehicle-treated group (n = 9), there was no significant change in hypotension and bradycardia response throughout the experiment.

into the ventricle, arterial pressure and heart rate started to decrease gradually. Mean arterial pressure was reduced to the lowest value of 66 ± 3.6 mmHg 1 h after injection, and then it was restored to the initial level by the third hour. Heart rate was reduced to the lowest, 229 ± 10.6 beats/min, 10 min after injection and returned to the initial level within 30 min. In control of nine rabbits with intraventricular injection of the vehicle, neither arterial pressure nor heart rate changed significantly throughout the experiment.

2. Responses to carotid sinus nerve stimulation. Electrical stimulation of CSN induced hypotension and bradycardia. By doubling the rate of stimulation stepwise from 10 to 160 Hz, frequency-dependent decreases in blood pressure and in heart rate were obtained. When 6-OH-DA was given intraventricularly, the hypotension and bradycardia in response to CSN stimulation were significantly exaggerated; the maximum effect being acquired within 2 h after injection at any stimulus frequency used. With 20 Hz, it appeared at 1 h in blood pressure and at 2 h in heart rate, as shown in Fig. 1. The exaggeration subsided by the fourth hour. In contrast, the injection of the vehicle showed no influence on the responses to CSN stimulation.

3. Carotid occlusion response. On 20 rabbits, occlusion of the left common carotid artery for 20 sec was performed, resulting in an elevation of arterial pressure and a negligible change in heart rate. Following 6-OH-DA administration, the rise in blood pressure in response to the procedure was greatly suppressed until 2 h after injection, and restored to the initial level within 3 h.

II. Effects of 6-OH-DA given 24 hours before
In 11 rabbits given 500 μg/kg of 6-OH-DA intraventricularly 24 h before, mean arterial pressure under α-chloralose and urethane anesthesia was 82 ± 2.5 mmHg in the average and heart rate was 276 ± 9.0 beats/min. These were significantly different from those of 29 control
animals, of which mean arterial pressure was
93 ± 1.8 mmHg and heart rate 247 ± 5.3
beats/min (p < 0.005, and p < 0.01). The hypo-
tension and bradycardia induced by CSN
stimulation were not significantly different from
the control at any examined stimulus frequency.
However, the pressor response to carotid
occlusion in the rabbits treated with 6-OH-DA
was 7.4 ± 0.46 per cent of initial pressure, which
was significantly less than 11.4 ± 0.82 of
vehicle-treated animals.
III. Effects of intraventricular injection of
norepinephrine
It was considered that 6-OH-DA might release
norepinephrine (NE) initially when given into
the ventricle. This possibility was tested in the
experiment of administering exogenous NE intra-
ventricularly.
Two hundred μg of NE was needed to cause
the same extent of decrease in arterial pressure as
in the animal treated with 500 μg/kg of
6-OH-DA. In eight rabbits given NE, the
bradycardia response to CSN stimulation was
enhanced and the pressor response to carotid
occlusion was suppressed, being equivalent to the
responses in 6-OH-DA-treated animals. Whereas,
the hypotensive response to CSN stimulation was
not augmented, occasionally it was inverted into
pressor response.
IV. Effects of intraventricular injection of
phenolamine
Phentolamine mesylate in the dose of 300
μg/kg was administered intraventricularly 4 h
after the injection of 6-OH-DA and then CSN
stimulation was performed again. The hypoten-
sion-bradycardia response to the stimulation was
abolished by the treatment. Intravenous applica-
tion of the same dose of phentolamine did not
affect the response essentially. In four rabbits
pretreated with 6-OH-DA 24 h before, intravent-
ricular injection of phenolamine in a dose of
300–500 μg/kg induced a fall in blood pressure
and heart rate. The response to CSN stimulation
to CSN stimulation was diminished.
V. Norepinephrine content in the brain tissue
Endogenous NE in seven rabbits at 4.5 h after
the injection of the vehicle was 1.184 ± 0.213
μg/g in the hypothalamus, 0.427 ± 0.058 in the
pons-medulla, and 0.242 ± 0.039 in the cortex.
By administration of 6-OH-DA, NE content was
significantly reduced 2 h after injection, and
reached to 21% of control in the hypothalamus,
14% in the pons-medulla and 47% in the cortex
4.5 h after (n = 8). In the rabbits pretreated with
6-OH-DA 24 h before, it was 28% of control in
the hypothalamus and 36% in the pons-medulla
(n = 9).

DISCUSSION
It is known that systemic administration of
6-OH-DA causes a rapid degeneration of
peripheral noradrenergic nerve terminals. Deple-
tion of NE is also obtained in the central nervous
system without reduction in peripheral organs
such as heart and blood vessels when 6-OH-DA is
given either directly into the brain tissue or into
the cerebrospinal fluid? In the present study, the
endogenous NE was markedly decreased in the
brain tissue, especially in the pons-medulla and
the hypothalamus. The areas where NE was
depleted are thought to have an important role in
the integration of inputs derived from carotid
sinus baroreceptor and the others.
In rabbits, electrical stimulation of CSN
induces hypotension and bradycardia and carotid
occlusion produces a rise in blood pressure. When
6-OH-DA was injected intraventricularly, the
resting arterial pressure and heart rate were
decreased and the responses to CSN stimulation
were augmented and the carotid occlusion
response was inhibited for 0.5–3 h. For the
explanation of mechanisms of these short term
effects of 6-OH-DA, may serve the experimental
data showing that exogenous NE given intraven-
ticularly could mimic the most of above-men-
tioned phenomena. The increase of NE release
immediately after 6-OH-DA administration, as
observed in the peripheral sympathetic nervous
system? may occur also in the central nervous
system and the released NE is conceived to
activate inhibitory pathways, resulting in a
reduction in resting arterial pressure, heart rate
and carotid occlusion response and an enhance-
ment of responses to CSN stimulation. This
explanation will be compatible with the hypo-
thesis that the antihypertensive drug of α-agonist,
clonidine, causes a long-lasting activation of the
central pathway of baroreceptor reflex?

The responses to CSN stimulation were not
different from those in the control at the time
when central NE was markedly decreased. In
these rabbits, intraventricular injection of an
α-receptor blocking agent, phentolamine, could
abolish or diminish the responses. This fact may
suggest that the central part of baroreceptor
reflex arc is related to noradrenergic neurons and
that the reflex can be functionally maintained by
a small remaining portion of them.
Recent investigations suggest that central NE plays an important role in the development and maintenance of hypertension of varied origins.1,10 Our study that baroreceptor reflex, which is indispensable to the regulation in hemodynamics, is not deteriorated by the depletion of central NE might give an insight into the concept of mechanisms of hypertension.

REFERENCES


Discussion:

Chairman: YUKIO YAMORI, Kyoto Univ.

Dr. YUKIO YAMORI: Since the late 1960's the role of central amines in blood pressure regulation has been gradually clarified, especially on the control mechanism of baroreceptor reflex, on the central hypotensive action of α-agonists and further on the pathophysiological role of monoaminergic mechanisms in various experimental hypertensions. Although the central administration of 6-OH dopamine is nowadays considered to be not an ideal tool to investigate the function of central noradrenergic neuron because of the diffuse and incomplete destruction of noradrenergic nerve terminals which leaves the noradrenergic receptors intact or even hypertensive, the analytical study of the present speakers throws some light on the role of noradrenergic neurones in central regulation of baroreceptor mechanisms.

Dr. MASAO IKEDA (Tokyo Univ.): I have two questions: (1) Does intraventricularly administered 6-OH dopamine deplete the peripheral norepinephrine? (2) Is the inhibitory effect of vagotomy on the response to carotid sinus stimulation related to the removal of not only the afferent but also the efferent components.

Dr. SHUICHI TAKISHITA (Kyushu Univ.): The dose of 6-OH dopamine intraventricularly administered in this experiment does not decrease the norepinephrine level of peripheral organs. The removal of efferent component is thought to be involved in the inhibition of the response to carotid sinus nerve stimulation by vagotomy.

Dr. YUSHIHIRO KANEKO (Yokohama City Univ.): Your experiment showed that the norepinephrine depletion in the brain or brainstem by 6-OH dopamine did neither raise the blood pressure nor affect the carotid sinus reflex. Does this finding deny the hypothesis that norepinephrine depletion in the brain is the cause of hypertension?

Dr. SHUICHI TAKISHITA (Kyushu Univ.): The functional differentiation of central noradrenergic neurons in blood pressure regulation was recently proposed by Dr. Yamori and other investigators. As the intraventricular injection of 6-OH dopamine depletes the central norepinephrine diffusely, we can not conclude that our data deny the pathogenetic involvement of central noradrenergic neurons in hypertension.

Dr. JUN FUJII (Asahi Life Insurance, Adult Disease Center): Can we understand that central catecholamine depletion by 6-OH dopamine does not affect the blood pressure level? How long did you follow up the blood pressure after the administration of 6-OH dopamine?

Dr. SHUICHI TAKISHITA: We observed the blood pressure so long as 10 days after the treatment. Although the number of our cases

Japanese Circulation Journal Vol. 39, May 1975
not enough for statistical analyses, we did not detect the blood pressure alteration. It is reported that blood pressure level did not change in rats with central norepinephrine depletion under chronic observation.

Dr. YUKIO YAMORI: As we reported, we observed the blood pressure for a long term in more than one hundred rats after the intraventricular injection of the various doses of 6-OH dopamine. After the acute stage of blood pressure fall due to the central sympathoinhibitory effect of released norepinephrine, blood pressure recovered to the initial level. However, in the rats after the intraventricular injection of relatively minute does of 6-OH dopamine, blood pressure sporadically increased or they showed a greater pressor response to immobilization stress. As Doba and Reis reported blood pressure increase after the specific destruction of nucleus tractus solitarii by 6-OH dopamine, more specific destruction of central noradrenergic depressor mechanism is necessary for the sustained rise of blood pressure.

Dr. HIROFUMI SOKABE (Jichi Med. School): I would like to hear Dr. Fukiyama’s comment on the inhibition of central inhibitory mechanism by angiotensin. Do you have any comment on the central depressor effect of β-blocker?

Dr. KOSHIRO FUKIYAMA (Kyushu Univ.): We think that the central pressor effect of angiotensin is due to the activation of sympathetic nervous system caused by the inhibition of baroreceptor reflex function. However, we have no idea on its relationship to the development of other forms of hypertension.

Dr. YUKIO YAMORI: At the 2nd Hypertension Conference we reported the augmentation of central pressor effect of angiotensin II after the intraventricular pretreatment of clonidine. There might be competition at the central noradrenergic receptor sites between angiotensin and α agonist, and the former might block the inhibitory effect of α agonist to release the vasopressor neurons. We suppose that the β mechanism of central catecholaminergic neurons is also involved in central blood pressure regulation and might be pressor in contrast to the α mechanism.

In conclusion of this discussion, the speakers offered some more evidences to support the involvement of central noradrenergic neurons in baroreceptor reflex. The recent progress in the studies on the central depressor mechanisms of α agonists substantiated the view for the existence of central α noradrenergic sympathoinhibitory or vasodepressor mechanisms. Although evidences for the involvement of central noradrenergic mechanisms in the pathogenesis of experimental hypertension are being accumulated, I think that we need more studies on this point, because not only the insufficiency of the noradrenergic inhibitory mechanisms but also the cooperation of some other mechanisms seem to be indispensable for the establishment of chronic hypertension.