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Increase in blood pressure by local injection of ketamine into the amygdala in rats

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Summary

To confirm that an increase in blood pressure induced by ketamine is mediated through the central nervous system, we examined the effect of ketamine, applied directly to the amygdala, on blood pressure. Six male Sprague-Dawley rats were used in the study. Under head-restrained and unanesthetized condition, 0.2 μl (5 mg/ml) of ketamine was injected in and around the amygdala at a flow rate of 0.2 μl/min through a glass pipette, and the blood pressure was recorded while monitoring the state of the animals by electroencephalogram and electromyogram. After ketamine injection, the injection site was marked by Pontamine Sky Blue infusion. Blood pressure was increased by ketamine injection into the basolateral and central nuclei of the amygdala, endopiriform nucleus and piriform cortex. In a total of 11 responses, an increase in blood pressure started with a mean latency of 193.5 ± 43.0 sec, reached its peak 180.2 ± 23.3 sec after the response onset, then gradually returned to the baseline with mean duration of 706.7 ± 113.5 sec. The mean fluctuation was 17.1 ± 2.5 mmHg. We revealed that blood pressure fluctuations induced by ketamine are associated with the amygdala. Elucidation of the mechanism of ketamine-induced blood pressure increase will lead to understanding of the mechanism of side effects of ketamine, and will contribute to its appropriate use.

Key words: ketamine, amygdala, blood pressure, N-Methyl-D-aspartate receptor antagonist, monoamine transporter
**Introduction**

Ketamine is known as a dissociative anesthetic that suppresses the cerebral cortex and activates the limbic system \(^1,^2\), and is unique as an anesthetic, since it induces blood pressure increase \(^3\). In recent years, many studies have been focusing on the antidepressant action of ketamine \(^4\)–\(^6\). Its antagonistic effect on opioid tolerance formation is also attracting attention as a treatment for chronic pain \(^7\)–\(^9\). Furthermore, ketamine is used as a research model for schizophrenia because it induces schizophrenia-like symptoms \(^10\).

Ketamine inhibits N-methyl-D-aspartate (NMDA)-type glutamate receptors, resulting in suppression of excitatory neurotransmission by glutamate \(^11\), and also increases free catecholamines by inhibiting monoamine transporters, resulting in sympathetic nerve activation, bronchodilation, tachycardia, and pressor action \(^12\)–\(^13\). Since blood pressure increase induced by centrally-applied ketamine is suppressed under pentobarbital anesthesia \(^14\), and intravenous administration of ketamine did not increase blood pressure in patients with cervical spinal cord injury \(^15\), it is considered that the action of ketamine is mediated by the central nervous system; however, its mechanism and site of action remain unknown.

Central blood pressure fluctuations are caused by emotional changes such as anger or fear, and such fluctuations are regulated by the amygdala \(^16\)–\(^18\). In clinical use, ketamine is known to induce nightmares or vivid dreams \(^18\), which can be taken as a sign of an emotional change and are characteristics of rapid eye movement (REM) sleep. During REM sleep, large fluctuations in the autonomic nervous system including blood pressure increase occur. The amygdala is involved in blood pressure fluctuations during
REM sleep\textsuperscript{19).} Based upon these findings, we hypothesized that ketamine acts on the amygdala to cause blood pressure fluctuations, and examined, in unanesthetized rats, the effect of ketamine applied directly to the amygdala on blood pressure.

Materials and Methods

Animals

All experimental procedures and protocols in this study were approved by the Animal Experiment Committee of Fukushima University, Japan (no.2018-07). Six male Sprague-Dawley rats (8-16 weeks old, body weight: 290-530 g) were used. The animals were reared at a temperature of about 24°C, a humidity of about 30%, and a 12-hour light-dark cycle with onset of light at 8 am.

Surgical procedure and experimental protocol

Implantation of a blood pressure transducer was performed under intraperitoneal anesthesia with pentobarbital (50 mg/kg). Atropine (0.2 mg/kg) was administered intraperitoneally to prevent asphyxiation due to excessive salivation caused by the anesthesia. For local anesthesia, 2% lidocaine was applied to the skin incisions. The lower abdomen was incised and the tip of the catheter (diameter: 0.4 mm) of a telemetric pressure transducer (PA-C-20, Data Sciences International, New Brighton, USA) was inserted in the abdominal aorta of the rat, and then fixed by an adhesive. The body of the transducer was placed in the abdominal cavity. Gentamicin was applied to the incision, and penicillin was intraperitoneally administered for 5 days after the
operation to prevent wound infection.

One week after the implantation of the transducer, the rat was fixed to the stereotactic apparatus under the pentobarbital anesthesia (50 mg/kg, intraperitoneal), and screw electrodes (diameter: 1.3 mm) for recording electroencephalogram (EEG) signals were embedded in the skull just above the frontal lobe and the parietal lobe. A pair of wire electrodes (multi stranded steel wires) to record electromyogram (EMG) activity was inserted in the cervical muscle and passed from the neck to the head through the space under the skin. EEG and EMG were recorded to monitor the state of the animals. A U-shaped plate for fixing the rat to the stereotactic apparatus was placed and fixed on the skull with dental cement. After the operation, gentamicin was applied to the incision and penicillin was administered for 5 days.

About one week after the surgery, the experiment was performed. Ketamine (5 mg/ml) was placed in a glass pipette (tip diameter 30 µm), which was connected to a tube filled with Fluorinert (FC-3283, 3M, St. Paul, MN, USA), then to a micro syringe (10 µl, Hamilton, Reno, NV, USA). The rat was fixed to the stereotaxic apparatus using the U-shaped plate under slight anesthesia of diethyl ether inhalation. After the rat became quiet, the glass pipette was inserted into the coordinates of the amygdala obtained from Rat Brain Atlas of Paxinos and Watson [20], and ketamine (0.2 µl) was injected at a flow rate of 0.2 µl/min by a micro syringe pump (EP-60, Eicom, Kyoto, Japan). The pipette was moved using oil manipulator, and ketamine was applied every 500 µm in one insertion track. Because the baseline of blood pressure is different between sleep and waking, ketamine infusion was restricted only during the waking period, which was confirmed by desynchronization of EEG. Artificial cerebrospinal fluid (ACSF) was applied to the coordinates (two sites) where ketamine induced blood
pressure increase.

After the injection, a glass pipette containing 2% Pontamine Sky Blue (PSB) was inserted to the same coordinates and PSB was applied by passing a negative current (10-15 μA for 3 min) to mark the injection site.

After the experiment, the rats were deeply anesthetized with 25% urethane and perfused through the ascending aorta with 200 mL saline followed by 300 mL of 10% formalin. Sections of 50 μm thickness were made and stained with 0.1% neutral red (Fig. 1).

Data analysis

Data analysis was performed using Spike 2 software (Cambridge Electronic Design Ltd, Cambridge, UK). The average systolic blood pressure about 10 seconds before injection was taken as baseline. The highest systolic blood pressure observed after the injection was taken as peak. The difference between the base and peak was taken as blood pressure fluctuation (ΔBPF). Time of blood pressure increase was defined as the time when the blood pressure began to rise continuously. Blood pressure fluctuations larger than 5 mmHg and longer than 60 sec were considered stimulus induced-responses, since blood pressure fluctuations exceeding these values were not observed during quiet waking. Student’s t-test was used to analyze differences between the ketamine-injected group and ACSF-injected group.
Results

Figure 2 shows an increase in blood pressure induced by ketamine injection (Thick line in Fig. 2A) to the basolateral amygdala. The blood pressure increase started 332 seconds after the injection, reached its peak 89 seconds after the onset of stimulus induced-response, and continued for 724 seconds. During the period without ketamine injection, this kind of slowly developing and long lasting fluctuations was not observed. Even when continuous muscle activity occurred, which lasted for about 100 seconds, blood pressure change was not observed (a in Fig. 2A). The increase in blood pressure was accompanied by slight and continuous muscle activity, which started to occur about 50 seconds before the blood pressure increase (b in Fig. 2A). Similar muscle activity was observed when blood pressure was at the control level (c in Fig. 2A). Thus, it can be said that a slow rise in blood pressure after ketamine injection was not caused by muscle activity.

Figure 3A indicates an increase in blood pressure induced by ketamine injection to the endopiriform nucleus (Fig. 3C), while no effect was observed when ACSF was applied to the same site (Fig. 3B).

The effective sites of ketamine injection for inducing blood pressure increase were the basolateral and central nuclei of the amygdala, endopiriform nucleus and piriform cortex (Fig. 4). The injection sites were limited to the rostral half of the amygdala (between 1.8 mm and 2.8 mm posterior to bregma). Among them, ketamine injection in the central part (between 2.3 mm and 2.8 mm posterior to bregma) was more effectively induced blood pressure increase.

In total, 70 injections were made into 43 brain sites, and 11 positive responses
(increase in blood pressure) were obtained from 8 sites. Regarding the 11 responses, the increase in blood pressure started with a mean latency of 193.5 ± 43.0 (mean ± standard error) sec, reached its peak 180.2 ± 23.3 sec after response onset, then gradually returned to the baseline with a mean duration of 706.7 ± 113.5 sec. The mean fluctuation was 17.1 ± 2.5 mmHg (Table 1). There were no correlations between the injection sites and latency, duration, or blood pressure fluctuations.

Figure 5 shows the maximum blood pressure fluctuation (ΔBPF) every 5 minutes after injection of ketamine or ACSF. Ketamine induced a significantly larger increase compared to ACSF 5 to 10 minutes after the injection.

Discussion

In the present experiment, ketamine administered to the amygdala of conscious rats induced increases in blood pressure with a latency of about 4 minutes and a duration of about 8 minutes. These results suggest that the amygdala is involved in the blood pressure increases caused by ketamine. Although the heads of the rats were fixed, they sat quietly throughout almost the entire experiment, and displayed normal sleep-wake cycles; therefore, they did not seem stressed. The present results showed the central effect of ketamine on blood pressure increase, which is consistent with the results of previous studies. In addition, the latency and duration of blood pressure increase were similar to those obtained in the results of previous studies in which NMDA was administered to the amygdala to induce blood pressure increase. In the present study, the latency before blood pressure increase was long with considerable variations. This
may be due to the diffusion time of ketamine. The significant increase in blood pressure continued for 5-10 min after ketamine injection, which was shorter than that of general ketamine anesthesia. This is because the amount of ketamine injected into the brain (approximately 0.002 mg/kg), was smaller than that used for general ketamine anesthesia (1-2 mg/kg).

Blood pressure fluctuations are caused by emotional changes such as anger or fear, and emotional changes are regulated by the amygdala. Inhibiting amygdala neurons with muscimol, a GABA receptor agonist, suppresses blood pressure increase associated with restraint stress. So, the amygdala is involved in blood pressure fluctuations caused by emotional changes during wakefulness. On the other hand, the amygdala is involved in changes in the autonomic nervous system and blood pressure during REM sleep. So, it is considered that the activation of the amygdala by ketamine administration is closely related to the increase in blood pressure.

Ketamine administration is known to cause nightmares, which are characteristics of REM sleep. It is highly probable that ketamine, by acting on the amygdala, causes changes similar to those occurring during REM sleep.

One of the action mechanisms of ketamine is to block open channel of NMDA-type glutamate receptor and suppress the activity of high-frequency discharge neurons. However, it is unclear whether the effect of ketamine injected into the amygdala is excitatory or inhibitory. Administration of NMDA to the amygdala induced a similar response in blood pressure to that of ketamine. We also confirmed that glutamate injected into the amygdala increases blood pressure (unpublished observation). Thus, the increase in blood pressure may be mediated through activation of amygdala neurons. A recent study reported that intravenous administration of ketamine increases c-fos
protein levels (indicator of cell activation) in the amygdala. Ketamine antagonizes NMDA receptors on the synaptic terminals of GABAergic neurons that contact presynaptically on the terminals of glutamatergic neurons, and suppresses GABA release from the terminals of GABAergic neurons. Therefore, ketamine increases glutamate release by the removal from the suppression of glutamate release by the GABAergic neurons. The amygdala contains not only NMDA receptors but also AMPA receptors. Since ketamine antagonizes NMDA receptors, but not AMPA receptors, postsynaptic neurons may be activated through APMA receptors. On the other hand, ketamine acts as α and β adrenergic receptor agonists, activating the sympathetic nervous system, and resulting in bronchodilation, tachycardia, and pressor action. In addition, ketamine inhibits catecholamine transporter to increase free catecholamines in the synaptic space. In the rat cerebral cortex, infusion of ketamine through microdialysis facilitated an increase in noradrenaline release. Noradrenergic neurons in the locus nucleus project to the amygdala, and amygdala neurons have receptors that mediate excitatory responses, such as α1, β1; therefore, the increase in blood pressure induced by ketamine may be associated with an increase in noradrenaline release from the terminals of the noradrenergic neurons projecting to the amygdala. Descending neurons from the amygdala would activate the medullary vasomotor center (rostral ventrolateral medulla), by passing the hypothalamus, midbrain and parabrachial nucleus. Ketamine induced blood pressure increase in the present experiment would be due to activation of sympathetic nervous system, however there still remains a possibility that the ketamine effect is mediated through suppression of parasympathetic nervous system.

In recent years, many studies focusing on the antidepressant action of ketamine have
been reported. Ketamine produces rapid and sustained antidepressant effects in treatment-resistant patients with major depressive disorder 5). It has been considered that the antidepressant effects of ketamine are due to its antagonizing action on NMDA-type glutamate receptors in disinhibition of GABAergic transmission 4,25-28). Ketamine is also attracting attention as a treatment for chronic pain by its antagonistic effect on opioid tolerance formation 7-9). For patients with chronic pain who are long-term users of narcotics, its combination with ketamine may reduce the amount and side effects of narcotics. Since ketamine induces schizophrenia-like symptoms, it is also used as a research model of schizophrenia 10). Thus, the effects of ketamine other than anesthesia are attracting attention 45). Ketamine-induced blood pressure increase is a problem in hypertensive patients as a side effect, and long-term administration of ketamine may cause cardiovascular events 46,47). Elucidation of the mechanism of ketamine-induced blood pressure increase will lead to an appropriate use of ketamine to control its side effects. In the present study, we revealed that blood pressure fluctuations induced by ketamine are associated with the amygdala. Further studies are required to understand the systematic mechanisms, including other brain sites, of ketamine-induced increase in blood pressure.

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Conflict of Interest

The authors declare no conflict of interest.
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| Latency (sec) | Response onset to peak (sec) | Duration (sec) | Blood pressure fluctuation (mmHg) |
|--------------|-----------------------------|----------------|----------------------------------|
| Mean±SE      | 193.5±43.0                  | 180.2±23.3     | 706.7±113.5                     | 17.1±2.5                        |
| Range [min-max] | 26-420                     | 89-336         | 243-1334                        | 8.0-32.8                        |

SE standard error
Fig. 1. A representative photomicrograph showing an injection site
Blue spot indicates an injection site after infusion of Pontamine Sky Blue to the same coordinate with ketamine injection. (Color figure can be accessed in the online version.)
Fig. 2. Increase in blood pressure after ketamine injection to the amygdala during waking.

A, Time course of blood pressure change after ketamine injection. Thick line, Injection period; a, period before injection; b, c, periods accompanied with muscle activity; BP, blood pressure; EMG, electromyogram; EEG, electroencephalogram; B, Injection site of ketamine (closed circle). BL, basolateral nucleus of amygdala; BM, basomedial nucleus of amygdala; Ce, central nucleus of amygdala; Br, bregma; The number after Br represents distance posterior to the bregma (mm).
Fig. 3. Effects after injection of ketamine and ACSF to the same coordinate

Time course after injection of ketamine (A) and ACSF (B). C, injection site;
ACSF, artificial cerebrospinal fluid.
Fig. 4. Injection sites of ketamine and its effect on blood pressure

Red circles indicate where ketamine-induced blood pressure increase was observed; Crosses indicate where no blood pressure increase was observed; Aco, anterior cortical nucleus of amygdala; AStr, amygdalostrial transition area; BM, basomedial nucleus of amygdala; BL, basolateral nucleus of amygdala; Ce, central nucleus of amygdala; En, endopiriform nucleus; Pir, piriform cortex. (Color figure can be accessed in the online version.)
Fig. 5. Time course of blood pressure fluctuation (⊿BPF) after injection of ketamine or ACSF. Vertical axis denotes the maximum ∆BPF every 5 minutes (mean ± standard error) (mmHg). *, significant difference (p < 0.05) by Student’s t-test. n, Number of measurements.