Activation of subfornical organ neurons in rats through pre- and postsynaptic α-adrenoceptors

Eiko Honda, Kentaro Ono, Shinji Kataoka, and Kiyotoshi Inenaga

Department of Biosciences, Kyushu Dental College, Kokurakitaku, Kitakyushu, Japan

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Honda, Eiko, Kentaro Ono, Shinji Kataoka, and Kiyotoshi Inenaga. Activation of subfornical organ neurons in rats through pre- and postsynaptic α-adrenoceptors. Am J Physiol Regul Integr Comp Physiol 290: R1646–R1653, 2006.—The effects of noradrenaline (NA) and its analogs on subfornical organ (SFO) neurons in rat slice preparations were investigated by using whole cell patch-clamp recording. In the current-clamp mode, the application of NA at 10–100 μM produced membrane depolarization (63%, 17 responsive neurons/27 neurons tested) and hyperpolarization (22%, 6/27 neurons). In the voltage-clamp mode, NA application at 1–100 μM produced inward currents (69%, 42/61 neurons) and outward currents (23%, 14/61 neurons). These currents remained in the presence of TTX or both glutamate and GABA receptor antagonists. In most of the neurons (25/31 neurons) showing inward currents in the presence of NA, the membrane conductance was not changed by voltage ramps or hyperpolarizing pulse stimulation. Similar responses were obtained by the application of the α1-agonist phenylephrine. The phenylephrine-induced inward currents were inhibited by the α1-antagonist prazosin. The α2-agonist clonidine decreased the frequency of spontaneous GABAergic inhibitory postsynaptic currents (4/10 neurons). In addition, RT-PCR assay and immunohistochemical staining showed the existence of α1α-adrenoceptors in the SFO. The results suggest that SFO neurons in rats are activated postsynaptically through α1α-adrenoceptors and that the activation is enhanced by suppressing GABAergic inhibitory synaptic inputs through presynaptic α2α-adrenoceptors.

Patch clamp; slice preparation; noradrenaline

The subfornical organ (SFO), which juts ventrally from the hippocampal commissura into the third cerebral ventricle, is a circumventricular organ. The SFO receives various neural inputs from brain regions, whereas it lacks a blood-brain barrier and has an abundant vascular supply (for a review, see Ref. 14). From these properties, the SFO has been thought to possess various sensors for neurotransmitters and blood- and cerebrospinal fluid-borne substances, and subsequently to control body fluid balance and the cardiovascular system (for reviews, see Refs. 8 and 15). In fact, angiotensinergic, cholinergergic, and hypertonic activation of SFO neurons elicits water intake (23) and vascular responses, and increases vasopressin release from the posterior pituitary (11, 12).

Noradrenaline (NA), which is released from catecholaminergic fibers and the adrenal medulla, may be one of such bioactive substances affecting SFO neurons. The catecholaminergic neurons in the A1 and A2 areas of the medulla project directly to various brain regions, including the SFO (3, 10). The NA level in the region of the SFO is increased by hemorrhage, whereas it is decreased by an elevation in arterial pressure (26). The peripheral baroreceptor information may be transmitted from the nucleus of the solitary tract to the SFO through α-adrenoceptors (16, 25). Further, microinjection of NA into the SFO increases water intake (17). Thus NA at the level of the SFO may play an important role in the regulation of cardiovascular function and body fluid homeostasis. However, little is known about whether the SFO neurons themselves are directly affected by NA and which subtypes of NA receptors are involved in the activation of SFO neurons. As far as we know, there are no reports showing the presence of specific subtypes of adrenoceptors in the SFO. In this study, the effects of NA and their analogs on SFO neurons were examined using a brain slice preparation of rat and a whole cell patch-clamp technique and molecular biological and immunohistochemical techniques.

MATERIALS AND METHODS

The study design was approved by the institutional review board of Kyushu Dental College.

Slice preparation. The techniques of slice preparations were similar to those reported previously (32). Male Wistar rats (4–6 wk old) were anesthetized deeply with ketamine (250 mg/kg sc) and decapitated. Coronal slices of 300 μm in thickness were prepared in a cold bathing medium and preincubated in a superfusion medium at room temperature for at least 1 h before transfer to a recording chamber. After transfer, slices were submerged and perfused at 1.6 ml/min with bathing medium at 32°C.

Solutions and drugs. The pipette solution used in the microelectrodes contained (in mM): 140 K gluconate, 1 MgCl2, 1 CaCl2, 5 EGTA, 10 HEPES, 4 Na2ATP (pH 7.2 adjusted with KOH). The normal bathing and superfusion media used in this experiment contained (in mM): 124 NaCl, 3 KCl, 1.24 KH2PO4, 1.3 MgSO4, 2.1 CaCl2, 25.9 NaHCO3, and 10 glucose. For the low-Na solution, Trizma hydrochloride at 124 mM was substituted for NaCl. The Ca2+-free solution contained (in mM): 118 NaCl, 3 KCl, 1.24 KH2PO4, 10.2 MgSO4, 25.9 NaHCO3, 0.1 EGTA and 10 glucose. These bathing and superfusion media were oxygenated continuously with 95% and 5% CO2. The drugs used were noradrenaline, phenylephrine, clonidine, isoproterenol, prazosin, TTX, amino-5-phosphohexonopetonic acid (AP-5), 6-cyano-7-nitro-quinoxaline-2,3-dione (CNQX), and picrotxin from Sigma (St. Louis, MO), and KB-R7943 from Tocris Cookson (Ellisville, MO). The drugs were applied to the slices by superfusion from separate storage bottles containing medium to which they had been added.

Recordings and data analysis. Whole cell recordings were made as described previously (32). The electrodes were double-pulled (P-87; Sutter Instrument, Novato, CA) from borosilicate thin-glass capillaries (1.2 mm ID and 1.5 mm OD), and had a final resistance of 5–10 MΩ when filled with the pipette solution. The series resistances (<25...
and membrane capacitance were compensated and checked regularly during the recording. The recordings were discarded if significant changes occurred. The transmembrane current and potential were recorded with an Axopatch 200A amplifier (Axon Instruments, San Francisco, CA). To get the voltage-current relationships, the ramp commands of 80 (−100 mV to −20 mV) or 100 mV (−100 mV to 0 mV) were applied at a rate of 20 mV/s. Occasionally, to test a continuous change of membrane conductance during the drug application, a constant hyperpolarizing pulse stimulation of −20 mV and 200 ms was applied repetitively to the neurons. Potential values were corrected for the junction potential (−11 mV), which was measured. Signals were filtered at 2 kHz and digitized at 1 kHz with an analog-digital converter (MacLab/8, ADI, Castle Hill, Australia). The numerical data are given as means ± SE, and n represents the number of neurons tested. The analysis of synaptic currents was done at a time point when the responses were at the maximum. Use of an event-detection threshold, which was set at the beginning of the analysis and thereafter held constant, allowed events to be detected with recording segments of either 60 or 120 s in duration. An event-detection threshold of more than 5 pA was used in the present study. The Kolmogorov-Smirnov test was used to determine whether two distributions were different, using a criterion of P < 0.05. The Student’s unpaired t-test was performed for analytical comparison.

RT-PCR assays. The total RNA from the SFO was analyzed with a protocol similar to that reported previously (19). After making slices, the SFO was dissected away from other tissues under a stereomicroscope. Two samples of SFO regions pooled from every three rats were performed for the following RT-PCR assays. Total RNA was extracted using RNeasy Mini Kits (Qiagen, Hilden, Germany). Reverse transcription of the total RNA (50 ng) was performed in a final volume of 20 μl using oligo-dT12–18 primer (0.5 μg/μl) and RNasin (10 units, Takara Biomedicals, Tokyo, Japan) with semiscript RT kit (Qiagen). The PCR was performed with a thermal cycler (PCR Thermal Cycler Dice; Takara Biomedicals). The specific primers for each α1a–d-subtypes of α1-adrenoceptors that have been designed were used: α1a, α1m, and α1d (21), as well as α1c (5). The PCR was performed with a PCR buffer containing 10 pmol primers, 2.5 U Taq DNA polymerase (Takara Taq Hot Start Version, Takara Biomedicals) and each transcribed cDNA in a final volume of 50 μl. Single-strand cDNA products were denatured and subjected to PCR amplification (40 cycles). Each PCR cycle consisted of denaturation at 94°C for 20 s, annealing at 60°C for 30 s and final extension at 70°C for 35 s. Total mRNA (10 ng) was used as a negative control. Total mRNA of taste buds was used as a positive control, because the existence of the mRNA in the lingual epithelium containing the taste buds has been reported (6). The PCR products were separated by electrophoresis on 2% agarose gel and visualized by ethidium bromide staining.

Fluorescent double-immunohistochemistry for anti-α1-receptor and anti-PGP9.5. Fluorescent immunohistochemistry was performed as reported previously (18). Three adult Wistar rats (6–8 wk old) were used for the immunohistochemistry. They were deeply anesthetized with pentobarbital sodium (100 mg/kg ip) and perfused through the left ventricle with PBS (in mM): 137 NaCl, 2.68 KCl, 8.1 Na2HPO4, 1.47 KH2PO4, followed by 4% paraformaldehyde in PBS. The brain blocks were dissected out and immersed in the same fixative for 3 h. After cryoprotectant treatment with 10–30% sucrose in PBS, sections throughout the SFO were mounted on MAS-coated glass slides (Matsunami Glass, Osaka, Japan), and air-dried. After a brief wash in PBS, the sections were treated with 10 mM citrate buffer (pH 6.0) for 10 min in an autoclave at 121°C. This process was done to help disrupt protein cross-bridges formed by formalin fixation and expose antigen binding sites (9). After 30 min of incubation with 0.5% goat serum in PBS, the sections were washed twice in PBS and then incubated with anti-α1-receptor [rabbit, Affinity BioReagents (Golden, CO) PA1-047, diluted 1:100] overnight at 4°C. The sections were then incubated with Alexa Fluor 488-conjugated goat anti-rabbit IgG [Molecular Probes (Carlsbad, CA), diluted 1:200] for 2 h. Next, immunohistochemistry against the neuron-specific gene product PGP9.5 [antiserum (rabbit), Ultraclone diluted 1:1,000] was performed. This antibody was labeled with the Alexa Fluor 488 dyes by Zenon Rabbit IgG Labeling Kits (Molecular Probes). Then, the sections were incubated with the labeled antibody for 2 h. Finally, the sections were coverslipped with VectaShield (Vector Laboratories, Burlingame, CA). For immunohistochemical controls, the primary antibody was omitted, and the staining images did not show any signals (data not shown).

RESULTS

Membrane depolarization by NA. Application of NA (10 and 100 μM) depolarized 17 SFO neurons (63%) when investigated in the current-clamp mode (n = 27). The resting potential was clamped manually at −65 to −70 mV. (Fig. 1, Aa and b, 11/18 neurons were depolarized at 10 μM and 14/22 neurons at 100 μM). The responses were reversible and reproducible. Occasionally, the depolarization was accompanied by action potentials (Fig. 1Ab). Six of 27 neurons (22%) were hyperpolarized by NA at 10 (3/18 neurons) and 100 μM (3/32 neurons). The average amplitude of the hyperpolarization was −4.0 ± 0.6 mV at 10 μM and −5.3 ± 0.7 mV at 100 μM. The remaining neurons were not responsive to NA at 10 and 100 μM.

Inward currents by NA and phenylephrine. Next, to clarify the mechanisms underlying the NA responses, the effects of NA on SFO neurons were examined under the voltage-clamp mode (Fig. 2A). The membrane potential was held at −70 mV. The application of NA at 1–100 μM induced inward currents in 42 of 61 neurons (69%). The average amplitudes were −7.0 ± 3.3 pA at 1 μM (9/18 neurons), −32.1 ± 5.2 pA at 10 μM (19/34 neurons) and −55.0 ± 7.7 pA at 100 μM (28/43 neurons) (Fig. 2B). The amplitudes increased with the NA concentration in a dose-dependent manner. In other neurons...
NA induced outward currents (14/61 neurons, 23%), with average amplitudes of 11.5 ± 1.6 pA at 10 μM (9/34 neurons) and 15.5 ± 6.3 pA at 100 μM (6/43 neurons) (data not shown). We found no outward currents at 1 μM NA (0/17 neurons tested). The NA-induced inward currents were also observed in the presence of TTX at 10 (−14.7 ± 2.8 pA, 3/5 neurons) and 100 μM (−43.0 ± 8.6 pA, 10/12 neurons) (Fig. 2C). In one neuron at 100 μM, an outward current was observed. Spontaneous excitatory postsynaptic currents (sEPSCs) and inhibitory postsynaptic currents (sIPSCs) were occasionally observed in the present experiment. We already reported that sEPSCs were blocked by glutamate receptor antagonists, and sIPSCs were blocked by GABA receptor antagonists (7). With the coapplication of glutamate receptor antagonists (CNQX at 10 μM and AP-5 at 20 μM) and GABA receptor antagonist (picrotoxin at 50 μM), NA-induced inward currents were also observed (Fig. 2Cb − 28.8 ± 11.4 pA, n = 5). These observations suggest that the action of NA on SFO neurons is direct.

The conductance changes were examined with voltage ramps and/or constant hyperpolarizing pulse stimulation, before and during the application of NA (Figs. 3 and 4). In one group of neurons showing NA-induced inward currents, there were no clear conductance changes with voltage ramp stimulation (25/31 neurons, Fig. 3, Aa and Ba) and constant hyperpolarizing pulse stimulation (3/6 neurons). In the neurons showing no clear conductance changes, the two current-voltage relationships did not intersect over the voltage range studied (Figs. 3Ba and 4Bc). In the other group of neurons showing the NA-induced inward currents, the currents were accompanied with an increased membrane conductance (with voltage ramp stimulation, 6/31 neurons, Fig. 3Bb; with constant hyperpolarizing pulse stimulation, 3/6 neurons, data not shown). The reversal potential in the voltage ramp stimulation experiments was estimated to be −29.5 ± 2.4 mV (n = 6). As for the NA-induced outward currents, we did not analyze the mechanisms in the voltage ramp stimulation experiments further because the number of cells was too few and the amplitudes were too small to do so.

To identify the receptor subtypes involved in the NA-induced currents, we tested the α1-agonist phenylephrine. The application of phenylephrine at 10–100 μM induced inward currents (Fig. 4A) (−19.6 ± 4.2 pA at 10 μM, 8/19 neurons; −23.6 ± 9.4 pA at 30 μM, 4/6 neurons; −31.2 ± 6.2 pA at 100 μM, 19/38 neurons). In the same concentration range phenylephrine did not induce outward currents in any SFO neurons. In the presence of phenylephrine no clear conductance changes were observed with voltage ramp stimulation (12/12 neurons tested at 30 and 100 μM, Fig. 4Ba) and constant hyperpolarizing pulse stimulation (5/5 neurons tested at 30 and 100 μM, Fig. 4Bb). With the voltage ramp stimulation, the current-voltage relationships did not intersect over the voltage range tested (Fig. 4Ba), as was seen during the application of NA (Fig. 4Bc). It is worthwhile noting that five of the ten neurons that showed the increased conductance and had the reversal potentials in the application of NA showed no change of conductance and had no reversal potentials in the application of phenylephrine. Furthermore, the phenylephrine-induced inward currents were suppressed almost completely by the α1-adrenoceptor antagonist prazosin at 1 μM, although little recovery was observed 15–35 min after its application (Fig. 5Bb) in the five neurons tested. These results suggest that the NA-induced inward currents in SFO neurons are produced at least partly through postsynaptic α1-adrenoceptors.

The application of the α2-agonist clonidine and the β-agonist isoproterenol at 100 μM also induced small but appreciable inward currents in some SFO neurons (clonidine, 11/31 neurons, −23.3 ± 4.6 pA, Fig. 5A; isoproterenol, 15/40 neurons, −12.5 ± 2.1 pA). The same concentrations of clonidine and isoproterenol also induced outward currents in 3 of 31 neurons (8.6 ± 1.4 pA) and 8 of 40 neurons (9.7 ± 1.3 pA), respectively (data not shown). Low concentrations of clonidine at 10 (n = 4) and 30 μM (n = 3, Fig. 5A) did not induce any currents.

To analyze the mechanisms of inward currents with no change of membrane conductance, we speculated the involvement of Na⁺-Ca²⁺ exchanger in the phenylephrine responses because such inward currents were observed by adrenoceptor activation in striatal cholinergic neurons (20) and also by hypocretin application in septohippocampal cholinergic neu-
rons (31). We applied the Na\(^+\)-Ca\(^2+\) exchanger blocker, KB-R7943, at 30 \(\mu\)M to three SFO neurons, which showed phenylephrine-induced inward currents. However, the application of KB-R7943 did not change the phenylephrine-induced currents. Meanwhile, the phenylephrine-induced currents were reduced in a low-Na\(^+\) perfusion solution (by 38.7 ± 6.8%, \(n = 7\)) and a Ca\(^2+\)-free perfusion solution (61.0 ± 9.5%, \(n = 3\)).

Presynaptic effects of clonidine on spontaneous IPSCs. The effects of phenylephrine, clonidine, and isoproterenol at 30 and 100 \(\mu\)M on sEPSCs and sIPSCs were analyzed. As reported in our previous study (7) and shown in Fig. 2Cb, the sEPSCs were blocked by glutamate receptor antagonists, and the sIPSCs were blocked by GABA receptor antagonists, suggesting that spontaneously excitatory synaptic inputs are mainly glutamatergic, and spontaneously inhibitory synaptic inputs are mainly GABAergic. The sEPSCs were not influenced by phenylephrine (\(n = 8\)), clonidine (\(n = 6\)), and isoproterenol (\(n = 10\)) (Data not shown). In contrast, the sIPSCs were suppressed by clonidine (4/10 neurons tested, by 20–82%, Fig. 6) but were not affected by phenylephrine (0/10 neurons tested). Application of clonidine significantly increased the mean interevent interval between the sIPSCs, whereas it did not change the amplitude (Fig. 6C). Isoproterenol suppressed sIPSCs in only one SFO neuron (1/14 neurons tested).

RT-PCR assays and immunohistochemical staining. To investigate the existence of \(\alpha_1\)-adrenoceptors in the SFO, RT-PCR was performed with \(\alpha_{1a-c}\). PCR products with the expected length of \(\alpha_{1a-c}\) were detected in SFO tissues, but not that of \(\alpha_{1d}\) PCR products (Fig. 7A). Nucleotide sequence analysis of all PCR products revealed that these bands were made from fragments of respective \(\alpha_1\)-adrenoceptor subtypes. These suggest that \(\alpha_{1a-c}\)-adrenoceptor subtypes are expressed in the SFO tissues.

Double-immunohistochemical staining was performed for \(\alpha_1\)-adrenoceptor (green) and neuron-marker PGP9.5 (red) in SFO section. Figure 7B (bottom right) shows that almost all of
the cells were yellow-colored, suggesting expression of $\alpha_1$-adrenoceptor on SFO neurons.

**DISCUSSION**

The present study shows that adrenergic inputs, which may be neuronal and/or humoral, directly depolarize the membrane of rat SFO neurons, at least in part, through postsynaptic $\alpha_1$-adrenoceptors and potentiate their neuronal activities. The application of NA at 1–100 $\mu$M induced membrane depolarization in 65% and inward currents in 69% of the SFO neurons. These currents were observed in the presence of TTX or both glutamate and GABA receptor antagonists. The responses

![Fig. 4](image-url)

Fig. 4. The absence of conductance changes in the phenylephrine and NA-induced inward currents. A: consecutive currents recorded from a SFO neuron. A, a and b: phenylephrine(30 $\mu$M)-induced inward currents. Ac: NA (30 $\mu$M)-induced inward currents. Note that the amplitudes of the phenylephrine and NA-induced currents were similar. The deflections in A, a and c represent the voltage ramps (from –100 to –20 mV, for 4 s). Other deflections in Ab represent the repetitive hyperpolarizing pulses (–20 mV and 200 ms). The holding potential was –70 mV. B: current-voltage relationships for the voltage ramps in Ab and Ac, and current changes produced by the hyperpolarizing pulse stimulation in Ab. The current-voltage relationships in B, a and c show no change in the membrane conductance and did not intersect over the voltage range tested. Bb: absence of changes in the membrane conductance before, during, and after washout of phenylephrine at 30 $\mu$M.

![Fig. 5](image-url)

Fig. 5. Involvement of receptor subtypes in NA-induced inward currents. Consecutive current records from two SFO neurons are presented. A: application of phenylephrine and NA-induced inward currents, whereas isoproterenol and clonidine at 100 $\mu$M induced only a small response. The NA-induced inward current was mimicked by phenylephrine. B: inward current induced by 30 $\mu$M phenylephrine was suppressed by simultaneous application of 1 $\mu$M prazosin. The holding potential was –70 mV.
RT-PCR was performed using total RNA prepared from SFO tissue, and amplification products of the transcribed from total mRNA isolated from the SFO tissue. RT-PCR was performed using four different primer sets (SFO1a, SFO1b, SFO1c, and SFO1d) to amplify the expected product size of each reaction. The products were visualized by UV illumination after ethidium bromide staining. The expected product size of each reaction is listed below each line.

**Fig. 7. Existence of α1-adrenoceptor agonist phenylephrine were similar to those by NA and suppressed by the α1-adrenoceptor antagonist prazosin.** These electrophysiological data suggest the existence of α1-adrenoceptors in the postsynaptic membrane of SFO neurons. Furthermore, this evidence is supported by the RT-PCR analysis and fluorescent immunohistochemistry in the present study.

Adrenergic synaptic innervation to the SFO is mainly from the A1 and A2 areas of the medulla oblongata (3, 10). Tanaka et al. (27) demonstrated from electrical stimulation of the A1 area in the rat that adrenergic excitatory neuronal inputs to the SFO are mediated mainly through α-adrenoceptors (27) and suggested that the pathways may transmit information from peripheral baroreceptors (25). The results of microdialysis experiments suggest that the NA level in the region of the SFO changes with blood pressure (26). Thus it seems likely that the α-adrenergic pathway involved in the cardiovascular regulation of the SFO is neuronal. On the other hand, the SFO, which lacks the blood-brain barrier, can detect humoral levels in the plasma. It has been reported that the concentration of circulating NA raises up to 2.8 ng/ml, that is, 15 nM (4). But, it was under an unusual condition, such as pheochromocytoma. The concentration was about 67 times lower than the threshold concentration (1 μM) of bath application in the present study. Although it does not seem to lie in the range of concentration that elicits the detectable responses in vitro, we cannot completely neglect a possibility that NA released from the sympathetic nerve affects SFO neurons.

In addition to the cardiovascular regulation of the SFO by NA, it has been reported that microinjection of NA into the SFO induces water intake, suggesting the body fluid regulation of the SFO by NA (17). However, for this matter, it remains to be clarified which subtypes of receptors in the SFO are involved in the responses.

The application of phenylephrine induced inward currents with no change of membrane conductance in all neurons tested. Similar inward currents by NA and by hypocretin have been observed in striatal cholinergic neurons (20), although the former responses were elicited through β1-subtypes of adreno-

**Fig. 6. Clonidine reduces the frequency of the spontaneous inhibitory postsynaptic currents (sIPSCs) in a SFO neuron, without changes of their amplitude.** A: representative example of inhibition of sIPSCs by clonidine at 100 μM. A, top: current trace. A, bottom: rate meter record of sIPSC frequency. B: expanded current records in control (a), during application of clonidine (b), and in washout (c) in A. C: cumulative probability plots of sIPSCs amplitude (a) and interevent intervals (b) in control and during application of clonidine. The mean amplitudes and numbers of sIPSCs during a 90-s recording period were 7.9 ± 0.1 pA and 1,102 events in control and 8.0 ± 0.2 pA and 345 events in the presence of clonidine. Note that clonidine did not change the distribution of sIPSCs amplitudes significantly but that the distribution of interevent intervals was shifted toward longer intervals. The holding potential was −70 mV.
ceptors, and in septohippocampal cholinergic neurons (31), respectively. It has been reported that these inward currents were elicited by activation of the Na\(^+\)-Ca\(^{2+}\) exchanger. To test the involvement of the Na\(^+\)-Ca\(^{2+}\) exchanger in the present experiment, we used KB-R7943 at a dose shown previously to block this exchanger (31). However, KB-R7943 had no effects on the phenylephrine-induced currents. Therefore, the Na\(^+\)-Ca\(^{2+}\) exchanger may not be involved in the phenylephrine responses of SFO neurons. Next, we tested the phenylephrine responses in a low Na\(^+\) perfusion solution and a Ca\(^{2+}\)-free perfusion solution. Both solutions suppressed them, suggesting the involvement of Na\(^+\) and Ca\(^{2+}\) ions in the responses. Several studies have reported that K\(^+\) channels also are affected through α\(_1\)-adrenoceptor activation, in which the membrane conductance must be changed (1, 29). In this study, we could not specify certain ion channels involved in the α\(_1\)-adrenoceptor activation. Possibly, multiple ion channels may be simultaneously influenced during the responses.

We also found the reversal potentials of the increased conductance of the NA-induced currents in some SFO neurons was \(-29.5\) mV. Because such changes were not observed by phenylephrine stimulation, the responses of the SFO neurons might not be mediated only through α\(_1\)-adrenoceptors, but through other subtypes of adrenoceptors, or mediated through both α\(_1\) and other subtypes of adrenoceptors.

It has been reported that the α\(_2\)-agonist clonidine hyperpolarizes the membrane in the current-clamp mode or induces outward currents in the voltage-clamp mode, through activation of potassium channels, in neurons of several regions of the brain (2, 13, 24). In this study, the application of NA induced membrane hyperpolarization and outward currents, and clonidine induced outward currents in a small number of SFO neurons. This implies that adrenergic inhibitory inputs to the SFO may be mediated through postsynaptic α\(_2\)-adrenoceptors. Therefore, if an electrode such as a CsCl-filled electrode was used to delete the involvement of potassium channels in the responses, NA-induced inward currents might be larger than those seen in the present study. We also observed clonidine-induced-inward currents at the high concentration of 100 μM, while low concentrations of clonidine (10 and 30 μM) did not elicit any currents in the SFO neurons tested (n = 7). A report has shown that clonidine acts as an α\(_1\)-agonist in high concentrations (22). Therefore, if it were present, the action through α\(_2\)-adrenoceptors in the postsynaptic membrane of SFO neurons would be not so strong.

The results of Tanaka et al. (27) from electrical stimulation of rat A1 area also suggest that adrenergic neuronal inputs to the SFO are mediated partly through α\(_1\)-adrenoceptors. The action through β-adrenoceptors was inhibitory. In this study, the application of the β-agonist isoproterenol induced both inward and outward currents in half of the SFO neurons tested, whereas the mean amplitudes were not as large (for inward currents \(-12.5\) pA and for outward currents 9.7 pA). From this, we think that the actions through β-adrenoceptors are secondary in the present preparations.

We have reported that spontaneously active inhibitory synaptic inputs in the SFO are mainly GABAergic (7). Several recent studies show that GABAergic synaptic inputs are suppressed through presynaptic α\(_2\)-adrenoceptor activation in the hypothalamus (30) and in the olfactory bulb (28). In this study, we found that the α\(_2\)-adrenoceptor agonist clonidine reduced the frequency of sIPSCs without any change of their amplitude. This implies that the inhibitory action through α\(_2\)-adrenoceptors on GABAergic terminals in the SFO is presynaptic and subsequently potentiates activities of SFO neurons, as in the other brain regions (28, 30). In the present study, we used 100 μM clonidine. This concentration of clonidine was strong enough to activate α\(_1\)-adrenoceptors as mentioned before. However, because we did not observe any effects of phenylephrine at 100 μM on sIPSCs, it is reasonable to consider that α\(_1\)-adrenoceptors may be absent in the GABAergic terminals or may have no involvement in the functional GABA release. Further, we found no influence of phenylephrine, clonidine, and isoproterenol on sEPSCs. Here, it may be worthwhile noting that the spontaneously active excitatory synaptic transmission in the SFO in rat slice preparations is not affected by adrenergic analogs.

In conclusion, neuronal or humoral adrenergic inputs to the SFO in rats activate neurons directly through postsynaptic α\(_1\)-adrenoceptors and indirectly through presynaptic α\(_2\)-adrenoceptors, by suppressing GABA release from the nerve terminals. A combination of these presynaptic and postsynaptic actions by NA may enhance neural activities of SFO neurons.

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