EFFECT OF AMINO ACIDS AND MONOAMINES ON THE NEURONAL ACTIVITY OF SUPRACHIASMATIC NUCLEUS IN HYPOTHALAMIC SLICE PREPARATIONS

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Abstract—Influences of amino acids and monoamines on the single unit discharges of the suprachiasmatic nucleus (SCN) were investigated using hypothalamic slice preparations. Iontophoretic application of GABA inhibited 90% and taurine inhibited 40% of SCN neurons, while glycine inhibited only 4%. L-glutamate excited about 50% of the neurons. Serotonin, noradrenaline and dopamine inhibited 30, 22 and 26% of the SCN neurons, respectively. Amino acid effects were observed equally in both the ventrolateral part of the SCN and the remaining part of it, while monoamine effects were observed preferentially in the ventrolateral part of the SCN where the optic fibers and serotonin nerves terminated. These results suggest that amino acids and monoamines modulate the neuronal activity of the SCN through their direct effects.

Previous reports (1, 2) on in vitro hypothalamic slice preparations have discussed the circadian rhythm of suprachiasmatic nucleus (SCN) unit activity. The autoradiographic 2-deoxy-D-[14C]glucose method reveals a circadian rhythm in glucose utilization by the rat SCN (3). The circadian rhythms of adrenocortical secretion, body temperature and running activity are abolished by SCN lesion (4–6). These results suggest that the SCN may act as at least one of the central controls of circadian rhythm.

It has been reported that nerve terminals containing high levels of serotonin (5-HT) exist in the SCN (7–9). The levels of noradrenaline (NA) and dopamine (DA) are moderate in this nucleus (10).

The levels of 5-HT, DA, NA, glycine and \( \gamma \)-aminobutyric acid (GABA) (11–15) and the number of NA receptors (16) in the central nervous system show a diurnal rhythmicity. Moreover, 5-HT uptake in the sliced SCN shows a 24 hr rhythmicity (17), and circadian variations in the activity of tyrosine hydroxylase, tyrosine aminotransferase and tryptophan hydroxylase are also reported (18). These reports have suggested that these neurotransmitters and enzymes are likely to modulate the SCN neuronal activity.

However, no detailed electrophysiological study concerning these neurotransmitters has so far been reported. The purpose of the present study is therefore to investigate the effects of amino acids and monoamines on the single units of SCN neurons.

Materials and Methods
A total of 32 male and 23 female Wistar strain rats weighing 180–320 g were used. All animals were housed in plastic cages with food and water ad libitum and maintained under standardized conditions of temperature (23–25°C) and light (0700–1900).

For slice preparations, the animals were sacrificed between 0830 and 1530 on the day of use. Only one hypothalamic slice,
about 300 μm thick, which included SCN was then coronally sectioned using a vibratome. Each slice was preincubated for at least 30 min in Krebs solution, and then transferred into a recording chamber. The preparation was perfused continuously with Krebs solution at a rate of 5 ml/min. The composition of Krebs solution was the same as described in our previous reports (1, 19).

Seven barrelled iontophoretic micropipettes were filled with the following substances: 5-Hydroxytryptamine creatine sulfate (33 mM, pH 3.7), noradrenaline hydrochloride (0.1 M, pH 4.5), dopamine hydrochloride (0.1 M, pH 4.5), γ-aminobutyric acid (0.5 M, pH 4.0), glycine (0.5 M, pH 4.0), taurine (0.5 M, pH 8–8.5), baclofen (1 mM, pH 5.6), bicuculline hydrochloride (5 mM, pH 3.5), sodium L-glutamate (0.2 M, pH 7.0) and sodium chloride (0.2 M, pH 7.0). Overall tip diameter of the seven barrelled micropipettes was about 1 μm, and DC resistance of each pipette was 50–200 MΩ. Recording electrodes were filled with 2 M NaCl and saturated fast green (DC resistance, from 5 to 20 MΩ) and were cemented to the multibarrelled pipette with its tip extending 20 μm beyond the pipette tip. All materials except glutamate were applied with positive current ranging from 5 to 100 nA (average 20 nA) from a constant current device (20). In order to minimize the leakage of these substances when not in use, a 5–10 nA current opposite in polarity to that of the ejection current was applied to each drug-containing barrel (20). After an electrophysiological experiment, a small mark was made by the iontophoretic application of dye (10 nA, 20 min) from the recording electrode. Each slice was then perfused with 10% formalin and was cut into 100–150 μm sections by a vibratome and stained with neutral red for identification of recording positions. Single unit discharges which remained stable for at least 10 min were recorded as unit discharges per sec. Electrophysiological experiments were terminated within 4 hr between 0900 and 1700 after sacrifice, since in previous report (1), we found that the firing rate of SCN neurons was continuously high during the period from 2 hr after “light on” to 2 hr before “light off”.

Results

Histology

In Fig. 1, the area surrounded by a broken line in the right SCN showed the ventrolateral part of SCN (VL-SCN) and the other area was regarded as the remaining part (R-SCN). The arrow in the left SCN indicates an example of the recording position.

Electrophysiology

Five to 10 neurons were recorded from each slice, and 98 neurons were obtained from 32 males and 87 from 28 females, 101 neurons being from VL-SCN and 84 from R-SCN. A total of 185 spontaneous active neurons were used for the iontophoretic experiment. Neurons were considered to be “unaffected” if their firing rate was not altered by a substance ejected from the micropipette with an ejecting current of at least 100 nA. There were no sexual differences in the drug effects, and the population of neurons responding to drugs and the

Fig. 1. Histology of the recording position in the SCN. An arrow shows an example of the recording position. Calibration=150 μm.
potency of drug effects were independent of the recording clock time during the light period.

1) **Amino acids**: The effect of GABA was examined on 163 out of 185 neurons displaying spontaneous activities. Approximately 90% of 163 SCN neurons was inhibited by GABA application in a dose-dependent manner (Fig. 2A, B), while 4% of them was first excited and then inhibited by GABA (Fig. 2C).

Bicuculline application did not antagonize the inhibitory effect of baclofen, but clearly antagonized the effect of GABA (Fig. 2E). Approximately 50% of 145 SCN neurons was excited by glutamate (Fig. 2A, B, D), while 42% of 33 neurons was inhibited by taurine (Fig. 2D). A majority (96%) of 93 SCN neurons was unaffected by glycine (Fig. 2B, C).

Table 1 shows the effects of amino acids on SCN neurons. Ninety-three % of 91 VL-SCN neurons and 83% of 72 R-SCN neurons were inhibited by GABA, while about 40% of neurons in each part of the SCN was inhibited by taurine, but 50% was excited by glutamate. Amino acid effects were observed equally in both the VL-SCN and R-SCN neurons. No relationship between drug effects and the firing rate of SCN neurons was observed. The neurons responding to amino acids, appeared to be distributed evenly over the low (Fig. 2B) and high firing rates (Fig. 2E).

2) **Monoamines**: Serotonin inhibited about 30% of 120 SCN neurons in a dose-dependent manner (Fig. 3A, C). Noradrenaline and DA inhibited about 22% of 134 neurons and 26% of 94, respectively (Fig. 3B, C). These monoamines produced a long-lasting effect in comparison with amino acids. Table 2 showed the effects of monoamines on SCN neurons. Serotonin and NA respectively inhibited 36 and 26% of VL-SCN neurons.

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**Fig. 2.** Effects of amino acids on the SCN neuronal activity. A-D, effects of amino acids on unit discharges. E, effect of bicuculline on the inhibitory influence of GABA or baclofen. A, C, D, different cells recorded from VL-SCN; B, E, from R-SCN. The number in the figure indicates the current intensity (nA). Bar, current injection period. Bacl, baclofen; Bic, bicuculline; GABA, γ-aminobutyric acid, Glu, glutamate; Gly, glycine, NA; noradrenaline; Na, NaCl; Tau, taurine.
Table 1. Effects of amino acids on SCN neurons

| Drug effects (%) | VL-SCN (N) | R-SCN (N) | VL-SCN+R-SCN (N) | Total (N) | Spontaneous firing rate (discharge/sec.) (Mean±S.D.) |
|------------------|------------|-----------|------------------|------------|---------------------------------------------------|
| GABA             | ↑ 5.5 (5)  | ↓ 93.4 (85)| ↑ 4.3 (7)        | 163        | 6.8±1.7                                          |
|                  | ↓ 1.1 (1)  | ↓ 13.9 (10)| ↓ 6.7 (11)       |            | 8.5±3.0                                          |
| Taurine          | ↑ 7.7 (2)  | ↓ 42.3 (11)| ↓ 6.1 (2)        | 33         | 6.6±3.4                                          |
|                  | ↓ 50.0 (13)| ↓ 42.8 (3) | ↓ 51.5 (17)      |            | 6.7±2.4                                          |
| Glycine          | ↑ 4.2 (2)  | ↓ 4.4 (2)  | ↓ 4.3 (4)        | 93         | 5.9±1.5                                          |
|                  | ↓ 95.8 (46)| ↓ 95.6 (43)| ↓ 95.7 (89)      |            | 6.3±2.9                                          |
| Glutamate        | ↑ 49.4 (42)| ↓ 50.0 (30)| ↓ 49.7 (72)      | 145        | 6.7±0.4                                          |
|                  | ↓ 49.4 (42)| ↓ 48.3 (29)| ↓ 48.9 (71)      |            | 6.3±3.1                                          |

†, augmentation; †, reduction; --, no change in firing frequency of neuron. N, the number of neurons tested, written in parentheses. VL-SCN, ventrolateral part of SCN; R-SCN, remaining part of SCN (see legend in Fig. 1).

while each amine inhibited only 13 and 15% of R-SCN neurons, respectively. Dopamine inhibited 23% of VL-SCN and 27% of R-SCN neurons.

Monoamines used here, excluding 5-HT, inhibited or excited the SCN neurons regardless of the firing rate, while SCN neurons inhibited by 5-HT showed preferentially higher firing rate (7.0±2.7 Hz) than those unaffected (5.7±2.6 Hz) (P<0.05. Student's
Table 2. Effects of monoamines on SCN neurons

| Drug effects (%) | VL-SCN (N) | R-SCN (N) | VL-SCN+R-SCN (N) | Total (N) | Spontaneous firing rate (discharge/sec.) (Mean±S.D.) |
|------------------|------------|-----------|------------------|-----------|--------------------------------------------------|
| 5-HT             | ‡          | 4.9 (4)   | 0 (0)            | 3.3 (4)   | 120                                              | 8.6±1.6* |
|                  | †          | 35.8 (29) | 12.8 (5)         | 28.3 (34) |                                                  | 7.0±2.7* |
|                  |            | 59.3 (48) | 87.2 (34)        | 68.3 (82) |                                                  | 5.7±2.6  |
| NA               | ‡          | 3.7 (4)   | 0 (0)            | 3.0 (4)   | 134                                              | 8.6±1.6  |
|                  | †          | 25.9 (21) | 15.1 (8)         | 21.6 (29) |                                                  | 6.8±3.1  |
|                  |            | 70.4 (56) | 84.9 (45)        | 75.4 (101)|                                                  | 6.1±3.1  |
| DA               | ‡          | 11.3 (6)  | 0 (0)            | 6.3 (6)   | 94                                               | 6.1±3.2  |
|                  | †          | 22.6 (12) | 26.8 (11)        | 25.6 (23) |                                                  | 6.3±3.2  |
|                  |            | 66.1 (35) | 73.2 (30)        | 69.1 (65) |                                                  | 6.1±3.0  |

†, augmentation; ‡, reduction; -, no change in firing frequency of neuron. N, the number of neurons tested, written in parentheses. VL-SCN, ventrolateral part of SCN; R-SCN, remaining part of the SCN (see legend in Fig. 1). 5-HT, serotonin; NA, noradrenaline; DA, dopamine, *P<0.05 significant difference from the value of neurons unaffected by 5-HT (Student’s t-test). #P<0.05, significant difference from the value in R-SCN (χ²-test).

Table 3. Responses of given SCN neurons to 3 monoamines, i.e., noradrenaline (NA), dopamine (DA) and serotonin (5-HT), showing how each neuron was affected by all 3 monoamines

| Drug effects (%) | Recording sites |
|------------------|-----------------|
|                  | VL-SCN | R-SCN | VL-SCN+R-SCN |
| NA ↓, DA ↓      | 7.5 (4) | 5.1 (2) | 6.5 (6)      |
| NA ↓, 5-HT ↓    | 17.0 (9)** | 0 (0) | 9.8 (9)      |
| DA ↓, 5-HT ↓    | 3.8 (2) | 0 (0) | 2.2 (2)      |
| NA ↓, DA ↓, 5-HT ↓ | 9.4 (5) | 2.5 (1) | 6.5 (6)  |
| Total (N)        | (63) | (39) | (92)        |

Letters represent % of neurons; NA ↓ means depression by noradrenaline, etc. N, the number of neurons tested, written in parentheses. VL-SCN, ventrolateral part of SCN; R-SCN, remaining part of SCN (see legend in Fig. 1). **P<0.01, significant difference from the value in R-SCN (χ²-test).

Table 3 shows the comparison of the effects of three monoamines on individual SCN neurons. A total of 92 SCN neurons, 53 from VL-SCN and 39 from R-SCN, were examined. In 53 VL-SCN neurons, 9 (17.0%) were inhibited by both NA and 5-HT (Fig. 3B), while 4 (7.5%) and 2 (3.8%) were inhibited by either NA and DA or DA and 5-HT (Fig. 3D). Five out of 53 cells (9.4%) were inhibited by all three monoamines (Fig. 3C). In 39 R-SCN neurons, only 2 (5.1%) and 1 (2.5%) were inhibited by either NA and DA or all 3 monoamines. Thus, the neurons responding to at least 2 monoamines were found more in the VL-SCN (20 of 53 cells) than in the R-SCN (3 of 39 cells) (P<0.01, χ²-test).

Firing patterns and drug effects

The SCN neurons were divided into the following 2 types according to their firing pattern: regular firing (Type I, Figs. 2 and 3 A, B, C, D) and irregular firing (Type II, Fig. 3E). The inhibitory or excitatory effect of 5-HT was observed in 34 of 92 Type I neurons, but only in 4 of 28 Type II neurons. On the other hand, all compounds tested, excluding 5-HT, exhibited their actions evenly
on Type I and Type II neurons.

Discussion

The effects of amino acids and monoamines on single unit discharges of SCN neurons in hypothalamic slices were investigated using iontophoretic application. An inhibitory amino acid, GABA, inhibited the majority (90%) of SCN neurons, irrespective of their firing pattern. Taurine inhibited about 40% of the SCN neurons tested, while glycine suppressed only 4%. Blume et al. (21) found that iontophoretic application of GABA inhibited 95% of medial hypothalamic neurons in anesthetized rats, and that of glycine inhibited 80%. This data suggested that medial hypothalamic neurons are generally more sensitive to GABA than to glycine. From the present result and above-mentioned report, it is indicated that GABA but not glycine plays an inhibitory role in the neuronal activity of SCN. Taurine has a depressant effect on spinal, brain-stem, and cortical neurons (22). However, it has not been elucidated whether taurine was a true transmitter or a secondary substance for GABA, glycine and other amino acid neurotransmitters (22). Since the order of potency, as determined by the number of SCN neurons depressed/the number tested, is GABA>taurine>glycine, taurine may play an inhibitory role in SCN neurons. An excitatory amino acid, glutamate, excited about 50% of the SCN neurons tested, and this result is well consistent with that of medial hypothalamic neurons (23).

In the present experiment, 5-HT, NA and DA inhibited 30, 22 and 26% of SCN cells, respectively. This result is consistent with previous findings (24) except that the population of neurons which was inhibited by these amines was high: i.e., 70, 65 and 60% of tested SCN cells were inhibited by 5-HT, NA and DA, respectively. This discrepancy may be due to the difference in experimental conditions, the location of recording sites in the SCN and anesthesia. Oomura et al. (23) demonstrated that iontophoretic application of NA and 5-HT inhibited 30 and 20% of medial hypothalamic neurons, respectively, in anesthetized rats. The present result in agreement with the above report indicates that monoamines may play an important role in the regulation of neuronal activity in the SCN as well as in the medial hypothalamus.

The iontophoretic application of 5-HT inhibited the cells recorded from the VL-SCN more than those from the R-SCN, and 5-HT inhibited or excited the cells firing at a high frequency with a regular firing pattern. These findings suggest that, among monoamine containing cells, 5-HT neurons are likely to modulate the activity of neurons with a high firing rate and regular firing pattern in the VL-SCN where both optic nerves (25, 26) and 5-HT fibers preferentially terminate (7, 8).

Although the existence of both inhibitory and excitatory synapses in the SCN has well been documented by anatomical studies (27), whether the neurons firing with regular pattern are excitatory or inhibitory and whether they are the efferents from SCN or interneurons in the SCN has not been revealed. Therefore, the functions of amino acids and monoamines in the SCN were not decisively demonstrated. However, it was confirmed in the present study that monoamines and amino acids play an important role in the regulation of SCN neuronal activity through their direct actions.

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