Effects of Intraseptally Injected Dopamine and Noradrenaline on Hippocampal Synchronized Theta Wave Activity in Rats

Yoshiki MIURA, Tsugutaka ITO and Toshiaki KADOKAWA
Department of Pharmacology, Research Laboratories, Dainippon Pharmaceutical Co., Ltd.,
33-94 Enoki-cho, Suita, Osaka 564, Japan

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Abstract—To clarify the functional role of catecholamine in the septal area, we investigated the effects of dopamine (DA) and noradrenaline (NA) injected into the medial septum (MSN) on hippocampal synchronized theta wave activity (TWA) in immobilized rats. The injection of DA (1-4 μg) into the MSN dose-relatedly enhanced hippocampal TWA, i.e., an increase of the total power in 3-7 Hz bands and little modification of the peak frequency. An enhancing effect of DA on TWA was also observed by the application into the diagonal band, but not into the lateral septum, nucleus accumbens, and lateral ventricle, indicating a selective effect of DA on the MSN and diagonal band. Furthermore, the enhancing effect of DA was blocked with the systemic treatment of haloperidol. Apomorphine (2 μg) injected into the MSN also increased the total power. NA (1-4 μg) enhanced TWA; however, NA, unlike DA, increased the peak frequency without modifying the total power, and the effect was not blocked by haloperidol, suggesting that the effect of NA on the septo-hippocampal neurons is different from that of DA. Muscimol (0.1 μg) and baclofen (0.05 μg) injected into the MSN depressed TWA, as indicated by a decrease of the total power. These results suggest that DA and NA, when injected into the MSN, heighten the functional level of the hippocampus.

Hippocampal theta wave activity (TWA), consistently observed at the arousal and rapid eye movement sleep stages, has been demonstrated to be initiated by septo-hippocampal neurons (SHNs) (1, 2) which are mainly cholinergic (3, 4), originating from the medial septum (MSN) and diagonal band of Broca (DB). These SHNs are mainly regulated by GABA released from the interneurons in the septum and by dopamine (DA) and noradrenaline (NA) released in the septum from the nerve terminals of the several hypothalamic and brainstem aminergic cells (5). For example, the dopaminergic neurons in the A10 area and others are known to terminate in the medial aspects of the lateral septum and in the DB (6).

Recently, Costa et al. (7) reported in their review that activation of the dopaminergic neurons exerted, via the GABAergic interneurons in the lateral septum, an inhibitory influence on the SHNs, leading to a decrease in the turnover rate of acetylcholine (ACh) in the hippocampus. Allen and Crawford (8) reported that muscimol, when injected into the MSN, depressed hippocampal TWA and decreased utilization of ACh in the hippocampus. Based on the finding with ACh utilization, it seemed likely that dopaminergic activation in the septum depresses hippocampal TWA as in the case of muscimol. However, the relationship between the dopaminergic activation in the septum and hippocampal TWA has not been determined with the direct application of DA into the septum. Furthermore, it has been demonstrated that methamphetamine and apomorphine, when administered systemically, strengthen the TWA (9).

In the present study, to clarify the functional role of catecholamine in the septum, effects of DA and NA injected into the MSN were examined on the hippocampal TWA in immobilized rats. In addition, effects of intra-
Materials and Methods

Male Wistar rats, weighing 300–350 g, were used. The animals were housed in temperature (24±1°C) and humidity (60±5%)-controlled rooms with 12/12 hr light-dark cycles, and they were fed food and water ad libitum until the start of experiments. Under anesthesia with ether, rats were fixed on the back, and the trachea was cannulated. They were placed on a stereotaxic apparatus (Narishige type), immobilized by d-tubocurarine chloride (1 mg/kg, i.m.), and artificially ventilated with room air at a rate of 60 strokes/min. A concentric bipolar electrode, insulated except for the tip, was inserted into the dorsal hippocampus (A: 3.1, L: 2.5, H: 2.5) according to the atlas of König and Klippel (11). A reference electrode was placed in the neck muscle. EEGs were recorded on an ink-writing oscillograph (Nihon Kohden, RGJ-3004) and concomitantly on an FM magnetic tape recorder (Nihon Kohden, RMG-5104) for subsequent computer analysis. A stainless steel guide cannula (diameter 0.5 mm) was inserted into the MSN (A: 8.9, L: 0.0, H: -1.0), lateral ventricle (A: 8.6, L: 1.0, H: 0.6), right lateral septal nucleus (LSN) (A: 8.9, L: 1.0, H: -1.0), DB (A: 8.9, L: 0.0, H: -1.2) or right nucleus accumbens (A: 8.9, L: 1.0, H: -1.0) using routine stereotaxic procedures in each experiment. After completion of surgical procedures, animals were kept under the conditions for 60–90 min until the stable EEGs were obtained. After a 5 min pre-injection recording, the injection needle, connected by a polyethylene tube to a microsyringe (Telmo, 25 μl) filled with saline, was protruded to a depth of 0.5 mm beyond the guide cannula, and the drug (1 μl) was injected over 60–70 sec. During the injection, EEG recordings were stopped, and 10 to 15 sec after the injection, restarted. The needle was left for an additional 3 min to allow the solution to diffuse away from the needle tip. In some experiments, effects of the drugs were examined under the treatment of haloperidol. In this case, haloperidol (0.5 mg/kg, i.v.) was administered after the 5 min pre-injection recording; and 10 min later, the drugs were intraseptally injected. All wound edges and pressure points were infiltrated with repeated injections of procaine HCl. Injection of d-tubocurarine was repeated as necessary during the course of the experiments. Body temperature was maintained constant by using an infrared lamp (36.5±0.5°C).

Using a data analyzer (Nihon Kohden, ATAC-450), EEG power spectral analysis was performed on each epoch (4 sec) by means of a Fast Fourier Transformation (FFT), and the results were displayed at every one-min interval for 32 min (Fig. 1). Moreover, the power average analysis was performed at 0–10 Hz for a 200-sec period selected from pre- and post-injection (until 10 min after injection) recordings. Total power of the theta wave was calculated as the sum of relative power (mm) in each frequency cut off every 0.25 Hz between 3 and 7 Hz, and the drug effect expressed as a percentage of the pre-injection value. Peak frequency was determined as the frequency showing the highest power in the power average analysis graph (Fig. 1).

Drugs used in this experiment were L-noradrenaline (Nakarai), dopamine hydrochloride (Sigma), apomorphine hydrochloride (Macfarlan Smith), muscimol (Sigma), baclofen (Ciba-Geigy) and haloperidol (Dai-nippon). NA was dissolved in 1 N HCl, and the solution was adjusted to pH 7.3–7.5 with the addition of an adequate volume of 1N NaOH. The other drugs were dissolved in saline.

At the end of the experiments, the injection loci were determined by the intraseptal injection (1 μl) of saturated fast green dye solution. EEG data obtained from the animals which did not have a dye spot within the expected region were discarded for analysis. Differences from the control or pre-injection value that are statistically significant were determined using the Student's t-test.

Results

The hippocampal electrical activity in unanesthetized, immobilized rats was mainly

septally injected GABA mimetics, muscimol and baclofen were also examined. A preliminary report of these findings has been communicated elsewhere (10).
characterized by synchronized, regular waves. These waves, designed as theta wave activity (TWA), had a peak frequency of 4.27±0.17 Hz (mean±S.E.M. of 6 experiments). The injection of saline (1 μl) into the MSN did not affect the TWA: total power in 3-7 Hz bands and peak frequency observed after the injection of saline were 99.6±3.5% of the pre-injection value and 4.33±0.16 Hz (n=5), respectively.

Effects of intraseptal DA and apomorphine on TWA: The injection of DA (4 μg) into the MSN increased the amplitude of TWA (Fig. 1). The EEG power spectrum array showed that the DA-induced increase in relative power of TWA, observed within 1 min after the injection, was maintained over 20 min (Fig. 1A). The total power in 3–7 Hz bands was dose-relatedly increased at a dose range between 1 and 4 μg of DA (Figs. 1 and 2). However, peak frequency was little changed with DA. Apomorphine (2 μg) also increased the total power, but little changed the peak frequency, as in the case of DA (Fig. 2).

In order to determine if the effect of DA on TWA is specific in the MSN or not, DA was injected into the neighboring areas of the MSN. DA (2 μg) injected into the DB...
Fig. 2. Effects of dopamine and apomorphine injected into MSN on hippocampal EEGs in rats. Total power is expressed as a percentage of the pre-injection value. Peak frequency (Hz) shows the highest power in power average analysis, which is measured at pre- (□□□) and post-injection (□□□□) periods. Each column represents the mean±S.E.M. obtained from 4 or 5 separate experiments, respectively. Abbreviations: Sal, saline; DA, dopamine; Apo, apomorphine. Differences from the saline control (total power) and pre-injection value (peak frequency) that are statistically significant: *P<0.01, **P<0.001.

The injection of muscimol (0.1 µg) or baclofen (0.05 µg) into the MSN reduced the total power in 3–7 Hz bands, but little affected the peak frequency (Fig. 5).

Effects of intraseptal DA and NA on TWA in haloperidol treated animals: Intravenous injection of haloperidol (0.5 mg/kg) did not affect the total power in 3–7 Hz bands. However, the peak frequency was slightly decreased, and the decrease was maintained over 30 min. Under the conditions, effects of DA and NA were examined (Fig. 6). DA (2 µg) injected into the MSN neither affected the total power nor the peak frequency. On the other hand, NA (2 µg) increased the peak frequency. The total power was not modified with NA. The effects of NA were quite similar to those of NA alone.

Discussion

In the present experiments, the injection of DA into the MSN was found to dose-relatedly increase the total power (3–7 Hz)
of the hippocampal TWA without affecting the peak frequency. In addition, the haloperidol treatment was found to block the total power-increasing effect which was observed with intraseptally injected DA alone. These results suggest the possibility that DA activates the SHNs to enhance the TWA. The findings were confirmed with the injection of apomorphine, a DA agonist, into the MSN which showed quite similar enhancing effects on the total power without affecting the peak frequency of TWA. On the other hand, DA, when injected into the LSN, nucleus accumbens or lateral ventricle, neither affected the total power nor the peak frequency of TWA, suggesting that the total power-increasing effect of DA is specific for the MSN. Furthermore, DA, injected into the DB, increased the total power as in the case of the injection into the MSN. The differentiation between the MSN and DB is practically difficult in our methods, since the DB is very closed to the MSN. Possibly, DA injected into the MSN is considered to penetrate into the DB. The dopaminergic neurons that regulate the activity of the septal neurons are known to terminate in the medial aspect of the LSN and in the DB (6). Accordingly, the increasing effect, by the injection of DA into the MSN, of the total power is strongly suggested to be due to an interaction of DA with its receptors on the SHNs existing in the MSN and DB.

Costa et al. (7) reported that activation of the dopaminergic neurons terminating into the LSN exerted, via the GABAergic interneurons in the LSN, an inhibitory influence on the SHNs, leading to the decrease of turnover rate of ACh in the hippocampus. Robinson et al. (12) showed that the injection of haloperidol into the MSN of the rat increased the turnover rate of ACh in the hippocampus. These results, which suggest the reduced hippocampal function by DA in the LSN and MSN, were strikingly different from our results indicating that the dopaminergic activation in the MSN leads to the enhanced TWA, while that in the LSN shows little effect on the TWA. Durkin et al. (13) reported that injection of haloperidol into the
Fig. 4. Effects of noradrenaline injected into MSN on hippocampal EEGs in rats. Each column represents the mean±S.E.M. obtained from 4 separate experiments. Other explanations were the same as those in Fig. 2. Inserted figure shows typical EEG powers in pre- (line) and post-injection (column) periods of noradrenaline. Abbreviations: NA, noradrenaline. Differences from the saline control (total power) and pre-injection value (peak frequency) that are statistically significant: $P<0.05, \#P<0.01.

LSN increased the hippocampal choline uptake. In the present study, muscimol and baclofen injected into the MSN reduced the total power of the TWA. The result with muscimol was consistent with the findings of Allen and Crawford (8) reporting that the injection of muscimol into the MSN reduced the TWA together with the reduced utilization of ACh. Based on these evidences, it is reasonable that DA, when injected into the MSN, may activate directly the SHNs, since the enhancement of TWA was observed. Under these conditions, the utilization of ACh is expected to be enhanced due to the DA-induced direct activation of SHNs. However, at the present time, the relationship between the utilization of ACh and the TWA in the hippocampus remains obscure. In addition, an involvement of the non-cholinergic SHNs can not be denied in its activation.

NA injected into the MSN showed different enhancing effects on TWA as compared with those of DA. Namely, NA dose-relatedly increased the peak frequency of TWA. The increase was not blocked with the haloperidol treatment. When injected into the LSN, NA was without effects. NA has been detected in the MSN and LSN (14, 15). Namely, autoradiographic findings confirmed the existence of noradrenergic projections from the locus coeruleus to the MSN in the rat. Robinson et al. (12) reported that systemically administered amphetamine increased the turnover rate of ACh in the rat hippocampus, its action probably attributable to increased release of NA but not of DA. Accordingly, the increase, by intraseptally injected NA, in peak frequency observed in this study may be due to direct activation of the NA receptors on the cholinergic SHNs in the MSN. Furthermore, the difference of the effects between DA and NA on TWA may be explained by the hypothesis of Apostol and Creutzfeldt (16) that there exist three different units in the septal
cells, where there are A units that are indicators of the neuronal tonus in the septum, B units related to the modulations of the amplitude of TWA, and C units related to the frequency of TWA. Namely, it is speculated that the dopaminergic and noradrenergic neurons may regulate the SHNs by relating to the B and C units, respectively.

Some relations of the hippocampal functions to memory tasks have been reported. For example, lesions of the SHNs abolish the hippocampal TWA (2) and impair the performance of maze task (17) and T-maze alteration (18). The injection of muscimol into the MSN in doses that decrease the turnover rate of ACh in the hippocampus increases the response rate during extinction of a food-reinforced lever-press response (19) and depresses the TWA (8). Haloperidol, bilaterally injected into the LSN, accelerates the extinction of a conditioned reinforcement task in a Skinner box (13). The present results that DA and NA, injected into the MSN, may activate directly the SHNs to enhance TWA strongly suggest that the monoaminergic neurons which terminate in the MSN and DB heighten, via activation of the SHNs, some functional levels of the hippocampus to improve the memory tasks. It will be necessary to elucidate the functional role of the utilization of ACh in the hippocampus and its relation to the memory task.

References

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Fig. 6. Effects of dopamine and noradrenaline on hippocampal EEGs in rats under haloperidol treatments. Haloeridol (0.5 mg/kg) was injected intravenously. Ten min after haloperidol injection, either dopamine or noradrenaline (2 µg) was injected into the MSN. In these cases, the value obtained between 5 and 10 min after haloperidol injection was used as the pre-injection value. Each column represents the mean±S.E.M. obtained from 4 separate experiments. Other explanations were the same as those in Fig. 2. Abbreviation: Hal, haloperidol. Differences from the control and pre-injection value that are statistically significant: \#P<0.05.

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