DOPAMINERGIC INHIBITION FROM SUBSTANTIA NIGRA OF CAUDATE NEURONS ACTIVATED BY CORTICAL STIMULATION

Sakae FUJIMOTO, Masashi SASA and Shuji TAKAORI
Department of Pharmacology, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto 606, Japan
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Abstract—Studies were performed to elucidate the role of dopamine originating in the pars compacta of the substantia nigra (SN) on caudate nucleus (CN) neurons receiving input from the motor cortex using cats anesthetized with α-chloralose. Activation with cortical stimulation was observed in 50 CN neurons, five out of which were also excited by SN stimulation. Conditioning stimuli applied to the SN, 30 msec preceding the test stimulus to the cortex, produced a significant inhibition of spike generation upon cortical stimulation in 28 out of 45 neurons tested and did not affect the remaining 17 neurons. The mean spike number of the 45 neurons was significantly reduced with SN conditioning stimulation. When dopamine up to 200 nA was iontophoretically applied to CN neurons, there was a significant inhibition of the spike generation with cortical stimulation in 9 out of 16 neurons. The mean spike number of the 16 neurons upon cortical stimulation was significantly reduced during dopamine application. In addition, a close correlation was observed between the effects of SN conditioning stimulation and iontophoretic dopamine. These results suggest that dopamine derived from the SN produces an inhibition of CN neurons receiving input from the motor cortex.

It has been reported that stimulation of the substantia nigra (SN) produces inhibitory and/or excitatory effects on neuron activities in the caudate nucleus (CN). Kitai et al. (1) have demonstrated that the monosynaptic EPSP of the CN neurons with stimulation of the pars compacta of SN is mediated by dopamine originating in the SN. However, this conclusion has been questioned by Bernardi et al. (2) and Herrling and Hull (3), since dopamine iontophoretically applied to the CN produced a slow membrane depolarization concomitant with a decrease in firing without an increase in conductance of the CN neurons. The results observed by others that the spike generation of CN neurons with SN stimulation was inhibited by dopamine receptor blockers such as haloperidol, suggest that dopamine may mediate an excitatory response, although the blockers may also act on the presynaptic dopamine receptors in the CN (4–6). On the other hand, the inhibitory effect of SN stimulation on CN neurons has been obtained in spontaneous and glutamate-induced spike firing (7). In such studies, several investigators have observed the correlation between the effects of SN stimulation and iontophoretic dopamine (7, 8). The other reports have shown the existence of both inhibitory and
excitatory responses or the incidence of an EPSP-IPSP sequence in the CN neurons upon SN stimulation (9-13). Thus, it remains inconclusive whether or not dopamine derived from the SN acts on CN neurons as an inhibitory or excitatory transmitter.

While intracellular studies by Kocsis et al. (14) have demonstrated that CN neurons receive excitatory and convergent inputs from the cerebral cortex and SN, extracellular experiments by Feltz and Albe-Fessard (9) and Fujimoto et al. (15) have demonstrated non-convergent and excitatory inputs to the CN from the cortex and SN. The present experiment was an attempt to elucidate the role of dopamine originating in the SN on CN neurons activated by cortical stimulation.

MATERIALS AND METHODS

Twenty-five adult cats were used. After all surgical procedures, such as cannulation into the trachea and femoral vein and craniotomy, had been completed under ether anesthesia, the animal was anesthetized with α-chloralose (30 mg/kg i.v.) instead of the ether administration, and all wound edges and pressure points were locally anesthetized with 8% lidocaine spray repeatedly throughout the experiments. The animal was then immobilized with gallamine triethiodide (5 mg/kg/hr, i.v.) and respiration was artificially sustained. Body temperature was maintained at 36.5-37.5°C with a heating pad.

A concentric bipolar electrode was stereotaxically inserted into the SN (A: 3.3, L: 2.5, H: -4.5), according to the topographic map of Snider and Niemer (16), and bipolar silver ball-tipped electrodes were placed on the motor cortex ipsilateral to the recording site. The extracellular neuron activity in the head of CN (A: 16.0-18.0, L: 3.0-4.0, H: 2.0-8.0) was recorded using a glass-insulated silver wire microelectrode (an electrical resistance of approximately 1-2 MΩ) attached along a seven-barreled micropipette. The pipette was filled with 0.5 M dopamine hydrochloride (Sigma) and 2M NaCl, and the distance between the tips of recording electrode and micropipette was within 20 μm. The chemicals were ejected to the vicinity of the target neuron using a microiontophoresis programmer (WP-I, model 160).

Conditioning stimuli consisting of 4 train pulses (0.2 msec, 4-12 V, 250 Hz) were given every 1.6 sec to the SN at various intervals preceding the test stimulus to the motor cortex (C-T interval). At least 20 successive responses displayed on an oscilloscope (Nihon Kohden, VC-9) were photographed and simultaneously stored on magnetic tape. Statistical significance of the data was determined by the Student’s t-test. The recording and stimulating sites were marked by passing a direct current of 20 μA for 20 sec and histologically checked with cresyl violet stain. Other details of procedures have been previously described (15, 17).

RESULTS

Effects of SN conditioning stimulation on CN neurons: Stimulation of the motor cortex produced spike generation in 50 CN neurons with mean spike latency of 16.4 ±1.4 msec (range: 4.5-36.0 msec), as shown in Fig. 1A. The spike could not follow a high frequency stimulation over 50 Hz, indicating that the spikes were transsynaptically elicited by the stimulus. Out of the 50 neurons activated by the cortical stimulation, 5 were also excited by test stimulation of the SN. Spontaneous firing rate of the CN neurons responding to the cortical stimulation was below 4/sec.

When conditioning stimuli were applied to the SN 30 msec preceding the test stimulus to the motor cortex, the spike generation with the cortical stimulation was significantly (P<0.01) inhibited in 28 out of 45 CN neurons (Fig. 1B). The remaining 17 neurons remained unaffected with the SN conditioning
stimulation. The mean spike number of the 45 neurons tested was 1.61±0.14 without the SN conditioning stimulation, while the number was significantly reduced to 0.96±0.10 with the conditioning stimulation. Figure 2 represents the mean percentage changes in spike number of 22 CN neurons when the intervals between conditioning and test stimulation were varied. These neurons could have been successfully tested for the full time course. The inhibition was observed during 20–100 msec of the C-T interval. The inhibitory effect of the SN conditioning stimulation on CN neurons was unrelated to the spike latency of neurons with cortical stimulation (Fig. 3).

**Fig. 1.** Effects of conditioning stimulation of the substantia nigra (SN) and iontophoretic application of dopamine on spikes of a caudate nucleus neuron activated by stimulation of the motor cortex. In each row, spikes were serially recorded every 1.6 sec from the lower to the upper. A: control, B: with SN conditioning stimulation, C and D: with iontophoretic dopamine of 50 and 200 nA, respectively.

**Fig. 2.** Mean percentage changes in spike number of caudate nucleus neurons elicited by cortical stimulation at various conditioning and testing time intervals (C-T interval). The mean was obtained from 22 neurons, each of which was inhibited by conditioning stimulation of the substantia nigra at a C-T interval of 30 msec. The vertical bar of each point indicates the standard error.

**Fig. 3.** Relationship between the mean spike latency of caudate nucleus neurons (n=50) upon cortical stimulation and the mean percentage change in spike number of the neurons produced by conditioning stimulation of the substantia nigra 30 msec preceding the test stimulus.
Table 1. Relationship between effects of iontophoretic dopamine and conditioning stimulation of the substantia nigra (SN) on caudate nucleus neurons activated by stimulation of the motor cortex

| SN condition | Inhibition | Excitation | No change | Total |
|--------------|------------|------------|-----------|-------|
| Inhibition   | 6*         | 0          | 0         | 6     |
| Excitation   | 0          | 0          | 0         | 0     |
| No change    | 3          | 0          | 7         | 10    |
| Total        | 9          | 0          | 7         | 16    |

*Number of neurons

Effects of iontophoretic dopamine on CN neurons: When 100–200 nA of dopamine was iontophoretically applied to the immediate vicinity of the CN neurons recorded, the spike generation with cortical stimulation was inhibited in 9 out of 16 neurons (compare C and D with A in Fig. 1). The mean spike number of the 16 neurons was 1.75±0.15 before dopamine application, while the number was significantly (P<0.01) reduced to 1.15±0.15 during dopamine application (200 nA) for 60 sec. The correlation between the effects of SN conditioning stimulation and iontophoretic dopamine on the spike generation with cortical stimulation was observed in 6 out of 16 neurons (Table 1). Seven out of 16 neurons remained unaffected by SN conditioning stimulation or iontophoretic dopamine. None of the 16 neurons exhibited an excitation with these treatments.

DISCUSSION

In contrast to the observation by Kocsis et al. (14) who demonstrated the excitatory convergence of inputs to the CN neurons from the cerebral cortex and SN, the present study did not show such convergence of the inputs in most (45/50 neurons) of the CN neurons tested. These results were in accord with our previous report (15) and findings by Feltz and Albe-Fessard (9). The difference between the aforementioned results (14) and our previous and present findings is probably due to a difference in experimental conditions such as type of anesthesia, types of recording electrodes, extra- or intracellular recording, recording sites in the CN, etc.

Kocsis et al. (14) have also reported that the latency of CN neurons directly activated by cortical stimulation ranges from 2.8 to 12 msec. Since the latencies obtained herein ranges from 4.5 to 36 msec, the CN neurons excited by cortical stimulation are considered to receive both direct and indirect inputs from the motor cortex. However, the CN neurons with latencies of less than 12 msec are suggested to be directly activated by cortical stimulation and the CN neurons with latencies longer than 12 msec include neurons polysynaptically excited by stimulation via other structures such as the thalamus. It is suggested, therefore, that the inhibition by SN conditioning stimulation of the spike generation in the CN neurons with cortical stimulation takes place directly on the CN neurons with cortical stimulation takes place directly on the CN neurons with latencies of less than 12 msec, but did not result from inhibition of other structures. This conclusion is further supported by the results obtained with iontophoretic application of dopamine which inhibited the spike generation of the CN neurons activated by cortical stimulation. In addition, the results that both SN conditioning stimulation and iontophoretic dopamine pro-
duced an inhibition of spike generation of the same CN neurons with cortical stimulation, suggest that dopamine derived from the SN acts as an inhibitory transmitter on the CN neurons activated by cortical stimulation. Our results are in line with those obtained by other investigators that spontaneous and/or glutamate-induced spikes are inhibited by both SN stimulation and iontophoretic dopamine (7, 8). Since the CN neurons, which fired spikes with the latencies longer than 12 msec upon cortical stimulation, was also inhibited by SN conditioning stimulation, the possibility remains that dopamine from the SN may inhibit such CN neurons themselves and/or transmissions of the neurons in other structures such as the thalamus.

On contrary, it has been reported that SN stimulation produces spike generation of the CN neurons, and the spikes are blocked with iontophoretic application of dopamine receptor blockers, suggesting that dopamine acts on the CN neurons as an excitatory transmitter (4, 5). Together with our results and other reports, it is reasonable to assume, as suggested by behavioral and biochemical studies, that CN neurons may have two types of receptors for dopamine, one of which mediates inhibitory response and the other which mediates excitatory response (11, 18-22). It seems likely that inhibitory receptors may mainly be located in the CN neurons responding to cortical stimulation and excitatory receptors may exist in the neurons activated by SN stimulation but not by cortical stimulation. Of course, the possibility that the inhibitory effect of SN stimulation on CN neurons activated by cortical stimulation may be due to an activation of inhibitory interneurons in the CN, could not be completely excluded at the present time, since iontophoretically applied dopamine may have spread and acted on such interneurons. However, the fact reported by McLennan and York (23) that the spike generation of CN neurons with SN stimulation was blocked by iontophoretic dopamine, may be explained by the inhibitory action of dopamine on the receptor located on the presynaptic terminals of neurons originating in the SN, as described by Kebabian and Calne (20).

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REFERENCES
1) Kitai, S.T., Sugimori, M. and Kocsis, J.D.: Excitatory nature of dopamine in the nigrocaudate pathway. Exp. Brain Res. 24, 351-363 (1976)
2) Bernardi, G., Marciani, M.G., Morocutti, C., Pavone, F. and Stanzione, P.: The action of dopamine on rat caudate neurones intracellularly recorded. Neurosci. Lett. 8, 235-240 (1978)
3) Henling, P.L. and Hull, C.D.: Iontophoretically applied dopamine depolarizes and hyperpolarizes the membrane of cat caudate neurons. Brain Res. 192, 441-462 (1980)
4) Feltz, P.: Sensitivity to haloperidol of caudate neurones excited by nigral stimulation. Europ. J. Pharmacol. 14, 360-364 (1971)
5) Norcross, K. and Spehlmann, R.: Selective blockade of excitatory caudate responses to nigral stimulation by microiontophoretic application of dopamine antagonists. Neurosci. Lett. 6, 323-328 (1977)
6) Richardson, T.L., Miller, J.J. and McLennan, H.: Mechanisms of excitation and inhibition in the nigrostriatal system. Brain Res. 127, 219-234 (1977)
7) Gonzalez-Vegas, J.A.: Antagonism of dopamine-mediated inhibition in the nigrostriatal pathway: A mode of action of some catatonia-inducing drugs. Brain Res. 80, 219-228 (1974)
8) Conner, J.D.: Caudate nucleus neurones: Correlation of the effects of substantia nigra stimulation with iontophoretic dopamine. J. Physiol. 208, 691-703 (1970)
9) Feltz, P. and Albe-Fessard, D.: A study of an ascending nigro-caudate pathway. Electrophysiol. clin. Neurophysiol. 33, 179-193 (1972)
10) Hull, C.D., Bernardi, G. and Buchwald, N.A.: Intracellular responses of caudate neurons to brain stem stimulation. Brain Res. 22, 163–179 (1970)

11) York, D.H.: Possible dopaminergic pathway from substantia nigra to putamen. Brain Res. 20, 233–249 (1970)

12) Zarzecki, P., Blake, D.J. and Somjen, G.G.: Interactions of nigrostriate synaptic transmission, iontophoretic O-methylated phenethylamines, dopamine, apomorphine and acetylcholine. Brain Res. 115, 257–272 (1976)

13) Zarzecki, P., Blake, D.J. and Somjen, G.G.: Neurological disturbances, nigrostriate synapses, and iontophoretic dopamine and apomorphine after haloperidol. Expl. Neurol. 57, 958–970 (1977)

14) Kocsis, J.D., Sugimori, M. and Kitai, S.T.: Convergence of excitatory synaptic inputs to caudate spiny neurons. Brain Res. 124, 403–413 (1977)

15) Fujimoto, S., Sasa, M. and Takaori, S.: Inhibition from locus coeruleus of caudate neurons activated by nigral stimulation. Brain Res. Bull. 6, 267–274 (1981)

16) Snider, R.S. and Niemer, W.T.: A Stereotaxic Atlas of the Cat Brain. University of Chicago Press, Chicago (1961)

17) Sasa, M., Fujimoto, S., Igarashi, S., Munekiyo, K. and Takaori, S.: Microiontophoretic studies on noradrenergic inhibition from locus coeruleus of spinal trigeminal nucleus neurons. J. Pharmacol. exp. Ther. 210, 311–315 (1979)

18) Cools, A.R., Struyker Boudier, H.A.J. and van Rossum, J.M.: Dopamine receptors: Selective agonists and antagonists of functionally distinct types within the feline brain. Eur. J. Pharmacol. 37, 283–293 (1976)

19) Costall, B. and Naylor, R.J.: The hypothesis of different dopamine receptor mechanisms. Life Sci. 28, 215–229 (1981)

20) Kebabian, J.W. and Caine, D.B.: Multiple receptors for dopamine. Nature 277, 93–96 (1979)

21) Norcross, K. and Spehlmann, R.: A quantitative analysis of the excitatory and depressant effects of dopamine on the firing of caudate neurons: electrophysiological support for the existence of two distinct dopamine-sensitive receptors. Brain Res. 156, 168–174 (1978)

22) Tittler, M., Weinreich, P. and Seeman, P.: New detection of brain dopamine receptors with (3H) dihydroergocryptine. Proc. Natn. Acad. Sci. U.S.A. 74, 3750–3753 (1977)

23) McLennan, H. and York, D.H.: The action of dopamine on neurones of the caudate nucleus. J. Physiol. 189, 393–402 (1967)