*Pharmacology Biochemistry & Behavior*, Vol. 31, pp. 417–423. © Pergamon Press plc, 1988. Printed in the U.S.A. 0091-3057/88 $3.00 + .00

# Enhancing Effect of Taurine in the Rat Caudate Spindle. II: Effect of Bilateral 6-Hydroxydopamine Lesions of the Nigro-Striatal Dopamine System

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Received 7 December 1987

HASHIMOTO-KITSUKAWA, S., S. OKUYAMA AND H. AIHARA. *Enhancing effect of taurine on the rat caudate spindle. II: Effect of bilateral 6-hydroxydopamine lesions of the nigro-striatal dopamine system.* PHARMACOL BIOCHEM BEHAV 31(2) 417–423, 1988.—Bilateral injections of 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle of rats resulted in destruction of dopamine (DA) nerve terminals in the striatum. DA contents decreased to 16.8, 15.0 and 13.7% of control values on 3, 5 and 7 days after the lesions, respectively. The time course of the effect of 6-OHDA lesions on apomorphine (0.5 mg/kg, IV)-induced stereotypy was investigated as the index of the development of supersensitivity. Stereotypy was unchanged on 3 days, but was enhanced 5 and 7 days after 6-OHDA lesions. Therefore, the sensitivity of postsynaptic DA receptors for apomorphine did not change 3 days after 6-OHDA lesions, although the striatal DA was depleted. The effects of bilateral injections of taurine into the striatum on the rat caudate spindle were determined 3 days after 6-OHDA lesions. Taurine, at a dose of 30 μg, enhanced the spindle in sham-operated rats, but this enhancement was not seen after 6-OHDA lesions. Intravenous administration of apomorphine (0.5 mg/kg) to lesioned rats suppressed the spindle, and this effect was prevented by a lower dose (3 μg) of taurine. These results provide further evidence that taurine enhances the spindle, possibly by decreasing the activity of the nigro-striatal DA system at the pre- and postsynaptic sites.

Electroencephalography Striatum Dopamine receptors Taurine 6-Hydroxydopamine Neuroregulators

IN a companion paper (8), we reported that taurine (10–30 μg) microinjected into the bilateral striatum enhanced the caudate spindle. A lower dose (3 μg) which, per se, did not affect the spindle reduced the suppressing effects of apomorphine and methamphetamine. From these results, we attributed the mechanism underlying the effect of taurine to decreases in the activity of striatal dopamine (DA) neurons. For confirmation, we examined the effect of taurine on the caudate spindle, when presynaptic elements of the nigro-striatal DA system were denervated by 6-hydroxydopamine (6-OHDA).

Taurine is known to inhibit the release of neurotransmitters at synapses and to modulate neuronal activity in the central nervous system (14). DA release was also reduced by taurine (1, 3, 7, 13), whereas the effects of taurine on the postsynaptic site of DA neurons have not been described so far. Koskimies and Ahtee (13) found that taurine antagonized amphetamine-induced locomotor stimulation, but did not alter the apomorphine-induced one. In our studies, we found that taurine (3 μg) reduced the suppressing effect of apomorphine, as well as methamphetamine (8). These findings suggest that taurine acts on the postsynaptic site of DA neurons. Whether or not taurine (3 μg) affects the suppression of caudate spindle induced by apomorphine after denervation of DA nerve terminals was thus examined.

## METHOD

### *Animals*

Male Wistar rats, weighing 250–300 g, were used. They were housed in groups of 3/cage in a light (12 hr cycle) and temperature controlled room with ad lib access to food and water.

### *6-OHDA Lesions of the Nigro-Striatal DA System*

Animals were anesthetized with sodium pentobarbital (40 mg/kg, IP) and fixed on a stereotaxic apparatus (Narishige,

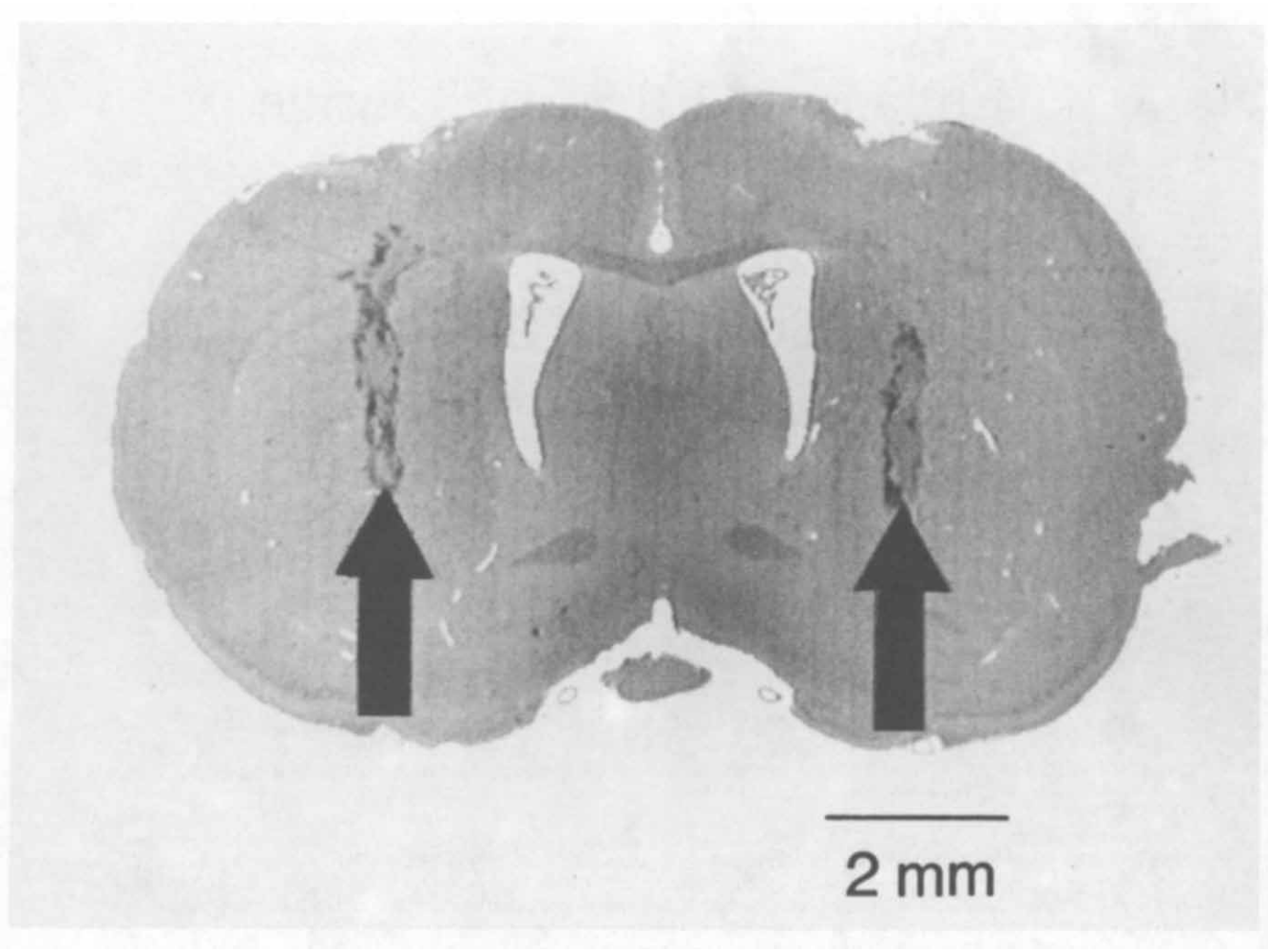


FIG. 1. Histological example of stimulating or injection sites (arrows) in the striatum.

SR-6). For 6-OHDA lesions of the bilateral nigro-striatal DA system, injection cannulae (o.d. 0.3 mm and i.d. 0.18 mm) were inserted into the bilateral medial forebrain bundle, using stereotaxic coordinates AP, 4.6; ML, 1.5; DV, 7.3 mm, according to the atlas of König and Klippel (12), and 8 $\mu$g/4 $\mu$l of 6-OHDA was injected at a rate of 1 $\mu$l/min. 6-OHDA was dissolved in saline solution containing 0.2 mg/ml ascorbic acid. Sham-operated rats received injections of vehicle instead of 6-OHDA solution. The injection cannula was left in position for 5 additional min. All rats were pretreated with desipramine-HCl (25 mg/kg, IP) dissolved in saline solution 30 min before 6-OHDA injections.

*Monoamine Assay*

The rats were killed by microwave irradiation (Toshiba, TMW-6402) and the brains were removed 3, 5, and 7 days after 6-OHDA lesions. The striatum was dissected using the methods described by Horn *et al.* (9). The regional concentrations of DA, noradrenaline (NA) and 5-hydroxytryptamine (5-HT) were determined by high-pressure liquid chromatography as described by Ishikawa and McGaugh (10). Each brain was weighed and transferred to a glass tube containing 0.025 N hydrochloric acid, 0.05 M EDTA and 3,4-dihydroxybenzylamine and was homogenized. The homogenate was transferred to a screw-capped glass tube containing butanol and solid sodium chloride, then shaken and centrifuged. The monoamines were extracted into 0.1 N hydrochloric acid. Fifteen microliters of the extract were injected onto a high-pressure liquid chromatograph (Yanaco, L-4000W) fitted with a voltametric detector (Yanaco, VMD-501) and Yanapak ODS column (25×0.4 cm i.d., Yanagimoto Manufacturing, Japan). All quantitations were based on the peak areas obtained for each of the substances compared with that for the corresponding internal standard.

*Stereotypy Induced by Apomorphine*

Stereotypy was assessed 3, 5, and 7 days after 6-OHDA lesions. Each rat was placed in an individual glass container (o.d. 18 cm) for at least 30 min before the start of an experiment. Rats pretreated with pargyline (100 mg/kg, IP, 30 min before) were given apomorphine (0.5 mg/kg) intravenously in a volume of 2.0 ml/kg body weight. Pargyline and apomorphine were dissolved in saline solution. Stereotypy was measured at each time, starting immediately after the administration of apomorphine, according to the rating scale of Costall and Naylor (5): 0) normal behavior; 1) exploratory

TABLE 1

CHANGES IN STRIATAL MONOAMINE CONTENTS ON DIFFERENT DAYS AFTER BILATERAL 6-OHDA LESIONS OF THE NIGRO-STRIATAL DA SYSTEM

| Days After Lesion | N | DA (μg/g wet tissue) | NA (μg/g wet tissue) | 5-HT (μg/g wet tissue) |
|---|---|---|---|---|
| 0 | 6 | 14.62 ± 0.49 | 0.24 ± 0.04 | 1.64 ± 0.06 |
| 3 | 6 | 2.45 ± 0.63* | 0.25 ± 0.03 | 1.86 ± 0.09 |
| 5 | 5 | 2.19 ± 0.69* | 0.33 ± 0.03 | 1.95 ± 0.10 |
| 7 | 6 | 2.01 ± 1.16* | 0.26 ± 0.04 | 1.44 ± 0.17 |

N: number of rats.
*$p<0.01$ vs. control values (day 0) (Dunnett's test).

TABLE 2

CHANGES IN STEREOTYPY INDUCED BY APOMORPHINE (0.5 mg/kg, IV) ON DIFFERENT DAYS AFTER BILATERAL 6-OHDA LESIONS OF THE NIGRO-STRIATAL DA SYSTEM

| Days After Lesion | N | Stereotypy Score Measured at the Following Times After Administration (min) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 5 | 15 | 30 | 45 | 60 |
| 0 | 6 | 1.3 ±0.2 | 2.2 ±0.2 | 2.5 ±0.2 | 2.0 ±0.0 | 0.2 ±0.2 | 0.0 ±0.0 |
| 3 | 6 | 1.0 ±0.0 | 2.3 ±0.3 | 2.0 ±0.4 | 2.3 ±0.3 | 1.5 ±0.2 | 0.2 ±0.2 |
| 5 | 6 | 1.2 ±0.2 | 2.5 ±0.2 | 3.2 ±0.2 | 2.8 ±0.2* | 2.5 ±0.3† | 0.5 ±0.2 |
| 7 | 6 | 1.3 ±0.2 | 2.8 ±0.3 | 3.0 ±0.3 | 3.0 ±0.3* | 2.7 ±0.2† | 0.5 ±0.3 |

N: number of rats. 0 min: immediately after administration of apomorphine. 6-OHDA lesions had significant effects on stereotypy 30 min, statistic value (3)=9.865, $p<0.05$ and 45 min, statistic value (3)=17.123, $p<0.001$, after administration of apomorphine (Kruskal-Wallis test). Levels of significance in comparison with control values (day 0): *$p<0.05$, †$p<0.01$ (Dunnett's test).

activity, discontinuous sniffing; 2) continuous sniffing; 3) continuous sniffing and discontinuous licking, biting or gnawing; 4) continuous licking, biting or gnawing.

*Caudate Spindle*

Three days after 6-OHDA lesions, rats were anesthetized with ether and immobilized with 2% gallamine triethiodide (0.2 ml/100 g body weight), because general anesthetics such as sodium pentobarbital influence the caudate spindle (4). Instead of that, all points of surgical and stereotaxic contact were infiltrated thoroughly with 8% lidocaine and anesthetic lidocaine ointment was supplemented every hour. Then animals were ventilated artificially, placed in a stereotaxic apparatus and subsequently subjected to surgical manipulations. The body temperature was maintained between 37 and 38°C, using a heating pad controlled from a rectal thermistor (Natsume, KN-474).

Screw electrodes were inserted through the cranium until their tips arrived at the dura overlying the frontal cortex. A cannula-bipolar electrode (o.d. 0.7 mm and i.d. 0.4 mm) that was capable of both electrical stimulation and injection of drug was implanted into the left striatum. A stainless-steel guide cannula was implanted into the right striatum and was fixed to the skull with small screws and dental cement. The tip of a cannula-bipolar electrode or guide cannula was located at AP, 7.3; ML, 3.0; DV, 3.5 mm; according to the atlas of Albe-Fessard *et al.* (2).

The caudate spindle was produced by electrical stimulation of the striatum (1 msec, 0.1 Hz, 5 V) and bipolar recordings from the frontal cortex were made. Sixty min later, measurements of the caudate spindle were started and after a further 10 min, drugs were administered. The intrastriatal microinjection was given through an injection needle (o.d. 0.3 mm and i.d. 0.18 mm) extending 1 mm beyond the tip of the guide cannula. Taurine was dissolved in saline solution. All injections into the striatum were given bilaterally at a rate of 1 μl/min using a microsyringe. The volume injected was 1 μl. The injection needle was left in position for one additional min.

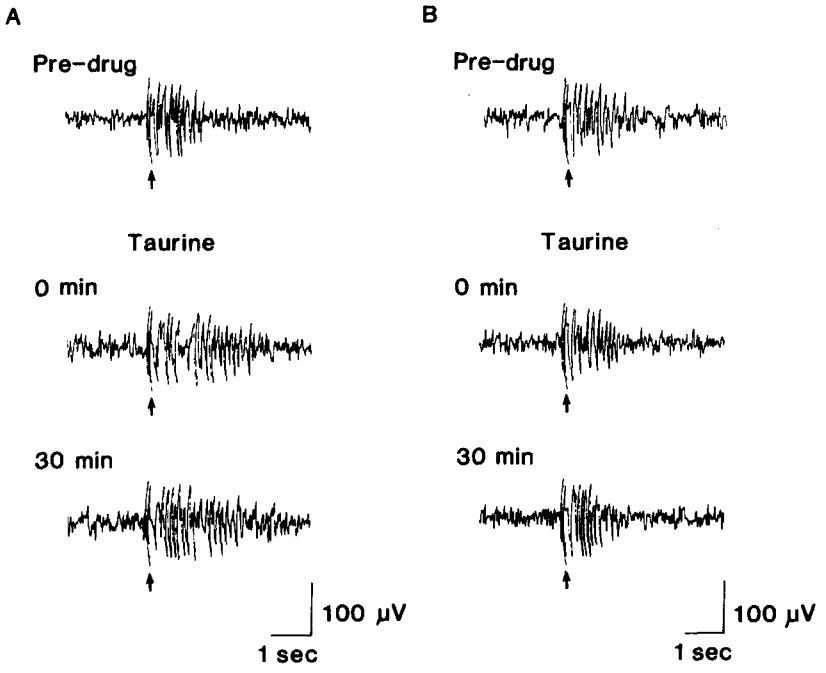


FIG. 2. Effect of bilateral 6-OHDA lesions of the nigro-striatal DA system on enhancement of the caudate spindle in rats induced by taurine (30 μg/side). (A) Sham operation. (B) 6-OHDA lesions of the nigro-striatal DA system. 0 min: immediately after administration of taurine. Arrows indicate stimulus artifacts.

TABLE 3

EFFECT OF BILATERAL 6-OHDA LESIONS OF THE NIGRO-STRIATAL DA SYSTEM ON ENHANCEMENT OF THE CAUDATE SPINDLE IN RATS INDUCED BY TAURINE (30 μg/SIDE)

| | | | Duration (percentage of predrug value) Measured at the Following Times After Administration (min) | | | | | |
|---|---|---|---|---|---|---|---|---|
| Treatment | Drugs | N | 0 | 5 | 15 | 30 | 45 | 60 |
| 6-OHDA | Vehicle | 6 | 83.7 ±6.5 | 95.3 ±3.0 | 94.3 ±4.9 | 99.1 ±2.3 | 101.5 ±10.2 | 103.9 ±5.2 |
| Sham | Taurine | 5 | 162.3 ±10.0 | 144.2 ±13.8 | 143.7 ±14.6 | 140.1 ±6.9 | 138.4 ±10.8 | 133.7 ±14.5 |
| 6-OHDA | Taurine | 6 | 101.4 ±12.3† | 96.7 ±7.7* | 117.7 ±6.7 | 119.4 ±5.0* | 114.5 ±6.5 | 118.4 ±7.2 |

The caudate spindle was measured 3 days after 6-OHDA lesions. Alteration in activity (index: duration) of the caudate spindle is shown as a percentage ratio of the predrug values.
N: number of rats. 0 min: immediately after administration of drug.
*$p<0.05$, †$p<0.01$ vs. sham-operated group (Dunnett's test).

In experiments to determine the effects of taurine on suppression of the caudate spindle induced by apomorphine, intrastriatal injections were given to pargyline-pretreated rats (100 mg/kg, IP, 30 min before) and 5 min later, apomorphine was given intravenously in a volume of 2.0 ml/kg body weight. Pargyline and apomorphine were dissolved in saline solution.

*Histology*

After the end of experiments, the location of the tips of the electrodes and guide cannulae were confirmed histologically. The animal was anesthetized with pentobarbital sodium and perfused with 10% formalin. Frozen 50 μm thick sections of the whole brain were cut on a freezing microtome (Komatsu, MA-101) and stained with hematoxylin and eosin (Fig. 1).

*Statistical Analysis*

Results are presented in terms of means±S.E.M. Calcu-

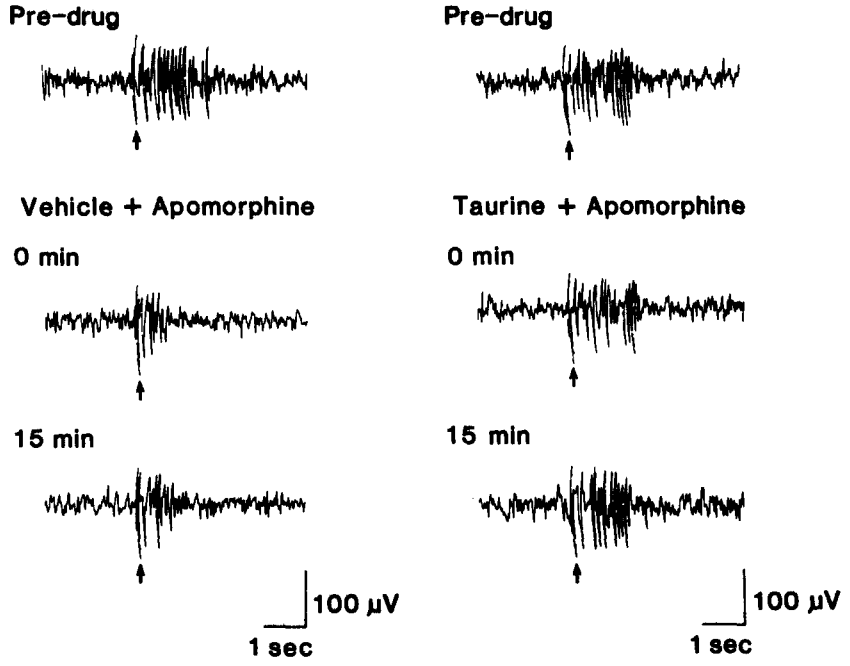


FIG. 3. Effect of taurine (3 μg/side) on suppression of the caudate spindle in rats with bilateral 6-OHDA lesions of the nigro-striatal DA system induced by apomorphine (0.5 mg/kg, IV). 0 min: immediately after administration of apomorphine. Arrows indicate stimulus artifacts.

lations including statistical analyses were performed on a VAX-8600 computer. Stereotypy data were analysed using Kruskal-Wallis test, followed by Dunnett's test for comparisons between 6-OHDA lesioned and control groups. Other data were analysed by ANOVA, and significant differences between groups were determined using Dunnett's test (6).

## RESULTS

### *Time Course of DA Loss in the Striatum and the Development of Behavioral Supersensitivity*

DA levels in the striatum fell to 16.8% of control values (day 0) 3 days after 6-OHDA lesions and remained at such levels for 7 days. In contrast, NA and 5-HT levels were unchanged (Table 1).

On the other hand, the stereotypy score 3 days after the lesions did not significantly differ from control values (day 0). Enhancement of stereotypy was apparent from day 5 (Table 2). Intense biting occurred in 5 of the 6 rats from day 5 to day 7, though only 2 or 3 of the 6 rats showed biting by day 3.

These results indicate that 3 days after 6-OHDA lesions, the DA content drastically decreased, but supersensitivity for apomorphine has not developed. The following experiments were performed 3 days after 6-OHDA lesions.

### *Effect of Taurine on the Caudate Spindle in Rats With 6-OHDA Lesions of the Nigro-Striatal DA System*

In lesioned rats, treatments with the vehicle led to no modification of the spindle.

Taurine (30 μg) enhanced the spindle in sham-operated rats. Immediately after taurine administration, the duration of the spindle was 162.3% of the predrug value.

This enhancement was prevented by 6-OHDA lesions. The value of the caudate spindle reversed to the level of vehicle-treated group (Fig. 2 and Table 3).

### *Effect of Taurine on Suppression of the Caudate Spindle Induced by Apomorphine in Rats With 6-OHDA Lesions*

Intravenous administration of apomorphine suppressed the spindle at the same dose (0.5 mg/kg) as that used to measure the stereotypy. This suppression was reduced by taurine at a lower dose (3 μg) (Fig. 3 and Table 4). This dose also reduced suppression of the spindle induced by apomorphine in the nontreated rats (8).

## DISCUSSION

To obtain evidence to support the hypothesis that taurine decreased the DA neuronal activity and enhanced the caudate spindle, we investigated the effect of taurine on the caudate spindle in rats with 6-OHDA lesions of the nigrostriatal DA system. Denervation of this system leads to a postsynaptic supersensitivity (16). This phenomenon enhances the sensitivity of postsynaptic DA receptors for apomorphine and thereby may alter the effect of taurine on the caudate spindle. However, supersensitivity has not developed 3 days after 6-OHDA lesions. Injections of 6-OHDA into the medial forebrain bundle led to a considerable fall in striatal DA levels without changes in the NA and 5-HT levels, but there was no significant increase in the apomorphine-induced stereotypy 3 days after the lesions. The time course of the stereotypy is similar to findings in biochemical results. Density of the DA receptors and DA-stimulated cyclic AMP accumulation did not increase on at least day 3 postlesion, although the striatal DA content sub-

TABLE 4

EFFECT OF TAURINE (3 μg/SIDE) ON SUPPRESSION OF THE CAUDATE SPINDLE IN RATS WITH BILATERAL 6-OHDA LESIONS OF THE NIGRO-STRIATAL DA SYSTEM INDUCED BY APOMORPHINE (0.5 mg/kg, IV)

| Treatment | Drugs | N | Duration (percentage of predrug value) Measured at the Following Times After Administration (min) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 0 | 5 | 15 | 30 | 45 | 60 |
| 6-OHDA | V + V | 5 | 94.1 ±5.3 | 109.0 ±6.2 | 109.3 ±6.3 | 102.1 ±11.5 | 99.0 ±5.6 | 100.4 ±13.0 |
| 6-OHDA | V + A | 6 | 57.8 ±5.0* | 65.3 ±4.4* | 68.8 ±5.5* | 94.5 ±11.4 | 85.5 ±7.4 | 68.2 ±5.9 |
| 6-OHDA | T + V | 6 | 92.4 ±4.9 | 98.4 ±4.6 | 105.4 ±4.8 | 105.0 ±7.5 | 102.2 ±7.5 | 100.4 ±9.5 |
| 6-OHDA | T + A | 6 | 86.8 ±6.4‡ | 91.5 ±6.0‡ | 110.2 ±6.6‡ | 129.4 ±10.3† | 126.5 ±10.3‡ | 132.0 ±10.9‡ |

The caudate spindle was measured 3 days after 6-OHDA lesions. Alteration in activity (index: duration) of the caudate spindle is shown as a percentage ratio of the predrug values. Rats pretreated with pargyline (100 mg/kg, IP, 30 min) were given taurine or vehicle 5 min before administration of apomorphine or vehicle. The caudate spindle was not significantly affected by treatment with pargyline and taurine.

V: vehicle. A: apomorphine. T: taurine. N: number of rats. 0 min: immediately after administration of apomorphine or vehicle.

*$p<0.01$ vs. V + V-treated group. †$p<0.05$ and ‡$p<0.01$ vs. V + A-treated group (Dunnett's test).

stantially decreased within 2 days (15). Taken together, these findings and our present data suggest that development of the supersensitivity has a slower time course compared with the decrease of DA levels. Consequently, determination of the effect of taurine on the caudate spindle was carried out 3 days after 6-OHDA lesions.

Since taurine (10–30 μg) enhanced the spindle dose-dependently in the nontreated rats (8), 30 μg of taurine was used in the present study. The enhancement of the caudate spindle induced by taurine was blocked by 6-OHDA lesions. Thus, the effect of taurine depends on neuronal activity of the DA system.

Other investigators suggested that taurine inhibits the release of DA and the firing rates of DA neurons (1, 3, 7, 13). The enhancement of the caudate spindle, as induced by taurine, can be explained by such a presynaptic effect. However, taurine also reduced the suppressive effect of apomorphine after 6-OHDA lesions of presynaptic elements of the DA system. This suggests that taurine affects the postsynaptic sites of DA neurons, in addition to its presynaptic effect. Izumi *et al.* (11) reported that taurine modulates the binding of $Ca^{2+}$ to microsomes isolated from the rat cerebral cortex, which is thought to regulate the postsynaptic $Ca^{2+}$ content, during depolarization and exerts suppressive actions on the central nervous system. Taurine may play such a role in regulating the nigro-striatal DA system.

In conclusion, our results suggest that taurine acts on both pre- and postsynaptic sites of the DA system, decreases neuronal activity and enhances the caudate spindle.

ACKNOWLEDGEMENT

We are grateful to M. Ohara (Kyushu University) for critical comments on the manuscript.

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