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Facilitation of Conditioned Motor Suppression by Microinjections of Dopamine in the Caudate Nucleus of Cats

I. ZARCO-CORONADO, C. REYES-VÁZQUEZ AND H. BRUST-CARMONA

Depto. de Fisiología, Div. de Investigación, Facultad de Medicina
Universidad Nacional Autónoma de México
México, D.F. Apartado Postal 70250

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ZARCO-CORONADO, I., C. REYES-VAZQUEZ AND H. BRUST-CARMONA. *Facilitation of conditioned motor suppression by microinjections of dopamine in the caudate nucleus of cats.* PHARMAC. BIOCHEM. BEHAV. **10**(5) 771–775, 1979.—The effects of Caudate Nucleus (CN) injections of catecholamines on the suppression of a motor conditioned response (MCR), lever pressing, was investigated. Cats were trained to press a lever to obtain 0.5 ml of milk when a conditioned discriminative stimulus was on (CS-on-reward on, MCR) and to suppress the response when the light was off (CS-off-reward off, suppression of motor conditioned response, SMCR). The bilateral application of 5 or 10 μg of Dopamine (DA) through chronically implanted cannulae in the CN significantly decreased the lever pressing in the non rewarding situation without changing the MCR. Injections of 5 μg of L-DOPA caused very small effects assessed during the following 10 min. However, 10 μg of L-DOPA produced a significant decrement of lever pressing (CS-off) in two out of four injections. These findings further support the postulation that catecholamines in the CN have a behavioral inhibitory action upon a motor conditioned response.

Motor conditioned responses Behavioral inhibitory actions of catecholamines Caudate nucleus Cholinergic and aminergic balance

THE Caudate Nucleus (CN) has been involved in modulating some types of behavior, specially those which involve somatic-motor activity [3, 10, 17]. Bilateral lesions in the CN affect learning requiring motor regulation [7,28]. The presence of high acetylcholinesterase concentrations in this nucleus [22,27] as well as of catecholamines, mainly dopamine (DA), has been histochemically demonstrated [1, 2, 11], and a balanced concentration of these chemical compounds has been proposed [14]. Hornykiewicz [20] has postulated that a balance between the cholinergic and dopaminergic neuronal activity is required for normal motor function.

Topical injections of acetylcholine (Ach) into the CN increase the probability of obtaining learned responses related to motor activity, while atropine microinjections decrease it [24]. Learned responses based on the inhibition of motor activity were not affected in the aforementioned study. Since in the autonomic nervous system the parasympathetic is balanced by the sympathetic responses, it was postulated that the catecholamines in the head of CN might be the main mediators in the acquisition of an inhibitory learned motor response. Subsequent tests indicated that the effects of norepinephrine topically injected into the CN resulted in an improvement in the inhibitory conditioned responses [9]. Bloom *et al.* [6] described inhibition in the unitary firing in CN by norepinephrine. However, as dopamine is highly concentrated in Corpus Striatum [4,5], and numerous DA receptors have been proposed to exist in this structure [16,28], and because DA has mainly inhibitory actions in the CN [12, 13, 23] it was reasonable to postulate that DA could be the natural neurotransmitter mediating the suppression of a learned motor response. To test this hypothesis in the present study, the effect of intracaudate bilateral microinjections of DA and of L-DOPA upon a food reinforced motor conditioned response (MCR), as well as upon the suppression of the MCR was determined.

METHOD

Fifty seven cats (2.5–3.5 kg of body weight) of either sex were trained to obtain 0.5 ml of milk each time they pressed a lever in a dimly illuminated Skinner type chamber (Lehigh Valley Electronics).

The time allowed for rewarded lever pressing was 12 min during which a luminous signal (discriminative stimulus CS) placed above the lever remained on (CS-on, MCR). The operant conditioning apparatus turned the light off for 1.0 sec at the end of each minute. This training pattern was repeated for 3 consecutive days. In the fourth session (day) after each 1 min period, the CS was turned off (CS-off) for 20 sec and no reinforcement was given for lever pressing during this short period (SMCR). Thus the length of the session was increased to a total of 16 min. Lever pressing in both situa-

tions was automatically recorded. After each session the cats received meat at a ratio of 35 g/kg of body weight. After 3 consecutive combined (CS-on and CS-off) sessions, the cats were anesthetized with pentobarbital (35 mg/kg IP) and placed in the stereotaxic apparatus. In 48 animals stainless steel cannulae (0.9 mm external diameter) were implanted in both caudate nuclei (A=16, L=4.5 and H=+4.5) according to the Jasper and Ajmone-Marsan atlas [21]. The remaining 9 cats were also anesthetized, placed on the stereotaxic apparatus but only the surgical procedures were performed (sham implantation). After that the cats were trained for 3 more days and on the following session a series of four bilateral injections was initiated, injection one every other day was made. The injections were performed introducing a stainless steel tube adjusted to reach the lower end of the cannula which was connected by polyethylene tubing to a microliter syringe. Eleven cats were injected with 5 μl of NaCl 0.9% (NaCl treatment); six cats were injected with 5 μg of DA dissolved in saline; six animals were injected with 5 μg of DA, but dissolved in water and 16 cats were injected with 10 μg of DA dissolved in water. In another group (n=4) 5 μg of L-DOPA were injected and finally in another (n=5) 10 μg of L-DOPA were applied. In all the cases the rate of the injection was 1 μl in 4–5 sec. The volume was always 5 μl. All the solutions were prepared immediately before application.

The effects of the microinjections as well as the sham injections on the conditioned responses were always tested after a 10 min period. After the injections all the cats received two more control sessions. Two days later all animals were perfused with 10% Formalin under deep nembutal anesthesia. To determine the site of the injection cannulae, histological sections were used as negatives to print photographic paper, according to the technique of Guzmán-Flores *et al.* [19]. The differences of lever pressing between a control series and a treatment series among the animals of a group was compared by the correlated *t* test. The differences between groups or treatments (NaCl, or 5 μg DA or 10 μg DA) was compared using F test and after that by the Student *t* test and the Dunnet test [30]. All the calculations were performed using a PDP 11/40 computer.

RESULTS

All animals learned to press the lever in 3–6 sessions and reached a mean of 6–10 lever pressings per min during the following 3 sessions. In the first session of response suppression all the animals diminished the number of lever pressings, but increased it during the second and third sessions in both experimental situations, MCR condition (CS-on-reinforcement on) and SMCR (CS-off-reinforcement off).

The sham implanted animals (n=9) diminished their pressing rate in the first session after the operation. Within 2–3 sessions they recovered their previous lever pressing rates showing just a few fluctuations during the remaining sessions (20 sessions). In the suppression condition the number of lever pressing also increased rapidly up to 25–35 per min, which were maintained during the following sessions (Fig. 1A).

The bilateral implantation of the cannulae also caused a decrement in the responses down to 1.5–2.5/min, but the pre-implantation rate recovered rapidly. Furthermore, the amount of lever pressing in the non-rewarding situation reached higher levels than in the sham implanted animals

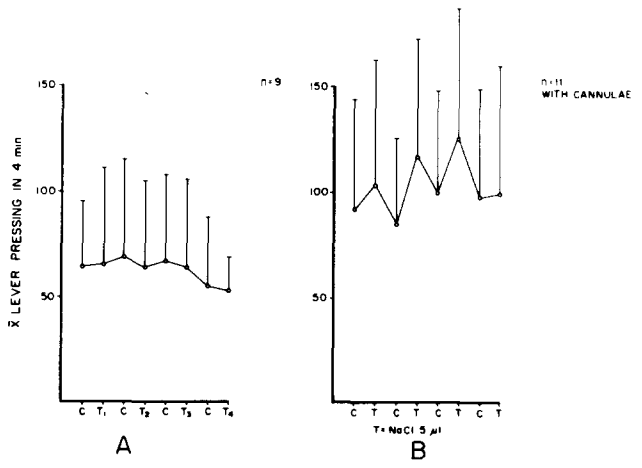

FIG. 1. Illustrates the average of lever pressing in the non-rewarding situation of control (C) and treatment (T) sessions. Right side represents the results of nine intact cats. Left side, animals with cannulae chronically implanted in the CN and treated with 5 μl of NaCl. Note the general increment of lever pressing after the implantation and after the first three treatments. In this and all figures a vertical line represents the standard deviation.

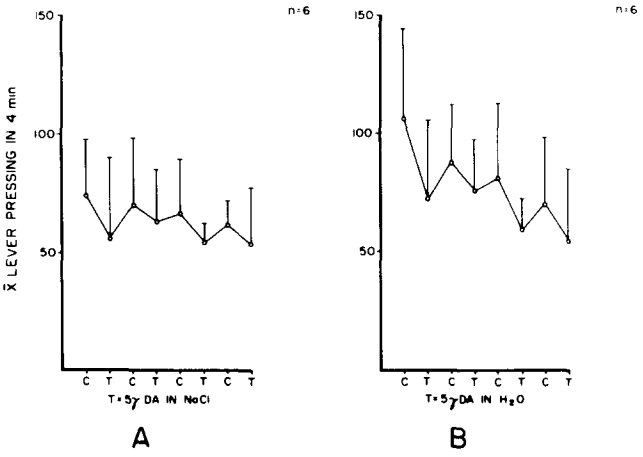

FIG. 2. Illustrates the average of lever pressing in the non-rewarding situation of control (C) and treatment (T) sessions. Note the tendency to decrease the lever pressing after the microinjection of 5 μg of DA dissolved in NaCl (right side) or distilled water (left side).

(Fig. 1B). However the difference is not statistically significant. The bilateral injection of saline solution in the CN without modifying the lever pressing in CS-on caused a clear increase in the situation of CS-off-reinforcement off. This tendency to increase was clearly observed in the first three injections (Fig. 1B).

In contrast, the injection of 5 μg of DA dissolved in NaCl produced a clear tendency to decrease the lever pressing rate during the suppression situation (Fig. 2A). However the difference compared with the control session is not statistically significant. Similar effects were also observed after the microinjection of 5 μg of DA dissolved in distilled water (Fig. 2B). The amount of lever pressing during the control ses-

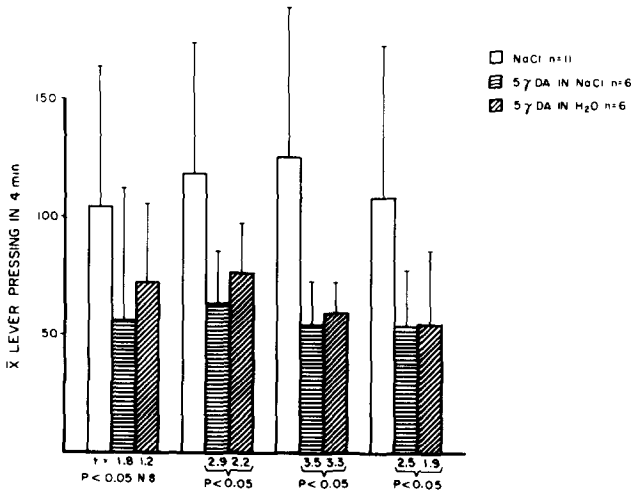

FIG. 3. The columns represent the average of lever pressing in the non-rewarding situation after each injection of NaCl (white column) or 5 μg of DA dissolved in NaCl (column with horizontal lines) or in water (column with diagonal lines) in both CN. Note the significant decrement of lever pressing after the DA application.

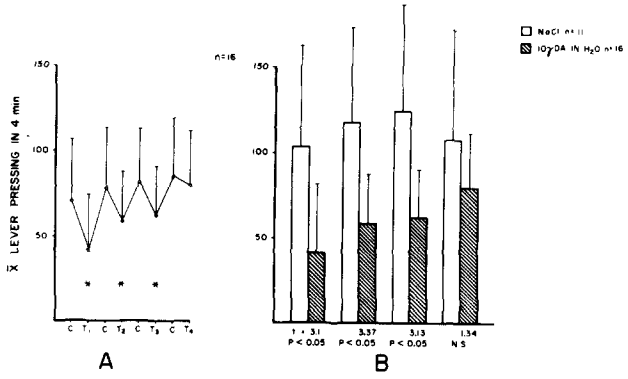

FIG. 4. The right side illustrates the average of lever pressing in the non-rewarding situation in the control (C) and treatment with 10 μg of DA session (T). Note the decrement of lever pressing which is statistically significant different (*) in the first three injections compared with control session. On the left side the columns represent the average of lever pressing during the four minutes following the injection in both CN of NaCl (white column) or 10 μg of DA (stippled bars). The differences are also statistically significant different.

sions of intact animals or of injected animals was very similar. But the amount of lever pressing always increased after each injection of NaCl and decreased after each one of the 5 μg DA application.

The injection of 10 μg of DA also produced a decrement of lever pressings during the non-rewarding situation. The F test comparing the amount of lever pressing after each treatment of NaCl or 5 μg of DA dissolved in NaCl, or 5 μg of DA dissolved in H_2O or 10 μg of DA showed statistically significant difference ($F=4.93$ for the first injection; 6.417 for the second; 7.502 for the third and 3.071 for the fourth one $p<0.05$). The student t test also gave a statistically significant difference at the level of 0.05 except for the first injection of 5 μg of DA dissolved in H_2O (Fig. 3). Finally, the Dunnet test showed that the differences of the amount of lever pressing in the non-rewarding situation was statistically significant ($p<0.05$) except for the first injection of 5 μg of DA dissolved in NaCl and the last one or 10 μg of DA. It is important to remark that the correlated t test also showed a statistically significant difference between the control series and the treatment series with 10 μg of DA (Fig. 4A). Naturally, the comparison of the decrement of lever pressing after 10 μg of DA and its increment, after NaCl injection showed statistically significant differences (Fig. 4B).

The injection of 5 or 10 μg of L-DOPA also produced a decrement in lever pressing in the non-rewarding situation, but only the second and third injection of 10 μg were statistically significant (Fig. 5). Once more it is important to mention that MCR did not change after any of these treatments. The histological sections showed that all the cannulae were located in the ventro medial anterior part of the head of the caudate nuclei.

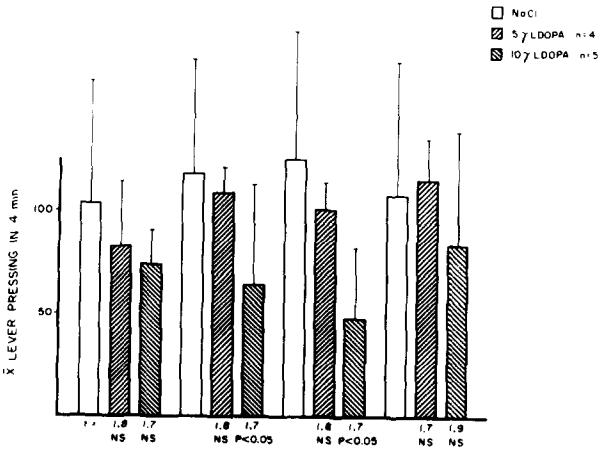

FIG. 5. The columns represent the average of lever pressing in the non-rewarding situation after the injection of NaCl (white) 5 or 10 μg of L-DOPA (stippled bars) in both CN. The diminution of lever pressing reached a statistically significant difference after the second and third injection with 10 μg.

DISCUSSION

Comparing the amount of lever pressings in the non-rewarding situation in intact cats with those of cannulae implanted cats seems to show an increment in the latter ones. However, the differences are not statistically significant. Subsequent to the saline injection MCR did not change but a trend to increase lever pressing in the non-rewarding situation occurred. Therefore, these data suggest that the cannulae implantations as well as the small injections produced small lesions, which are enough to change the inhibitory activity in CN. These effects have been described previously, and small lesions in CN have affected mainly the inhibitory mechanisms [7,8], and that only larger lesions also affect the facilitating mechanisms of lever pressings [25].

The topical application of 5 μg of DA produced a decrement of lever pressing in the non-rewarding situation (SMCR), without modifying the lever pressing rate (MCR) in the rewarding situation. Such data suggests an improvement

in the inhibitory process of MCR, despite the lesioning effect which also appear to occur. The amount of lever pressing after the 5 μg of DA injection is significantly lower than that obtained after the saline solution injection. Following the injection of 10 μg of DA the amount of lever pressing is lower than that obtained during control sessions. This significant difference was not observed after 5 μg of DA applications but it is important to note that the sample was increased in the case of 10 μg (n=16) as n=6 in each 5 μg group. It is possible that the effect observed with 5 μg would also be significantly different compared to control sessions if the number of cats were increased. As a matter of fact, we pooled together the amount of lever pressing of the two groups of 5 μg of DA and compared it with the control series using the correlated *t* test. The results indicated a significant difference for the first, second and third injection ($p<0.05$). In all cases the effect of 5 μg or 10 μg of DA is significantly different from the one observed after the injection of NaCl. The smaller effects of L-DOPA could be attributed either to a lack of uptake, transport and utilization by the striatal terminals or to its fast oxidation by MAO. It has been reported that L-DOPA applied without MAO inhibitors has no action [18].

These results lend further support to the hypothesis that DA could be the mediator of the behavioral inhibitory effects in the CN. In fact, it has been reported that the topical application of DA produces inhibition of both the evoked potentials in the CN [23] and of behavior [15]. Furthermore, recent data on the effects of 6 hydroxydopamine (6-OHDA) injections in the CN agree with this conclusion [26].

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