EFFECTS OF INTRACEREBRAL ADMINISTRATION OF ATROPINE AND MORPHINE ON THE CAUDATE STIMULATION-INDUCED CAUDATE SPINDLE IN RATS

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The electrical stimulation of the caudate nucleus could induce a caudate spindle in the rat as in case of the cat and monkey. The spindle was enhanced by atropine (3–10 mg/kg, *i.v.*) or morphine (3–10 mg/kg, *i.v.*), and the effects of these drugs were completely abolished by eserine (0.3 mg/kg, *i.v.*), but not by methysergide (1 mg/kg, *i.v.*), PCPA (400 mg/kg, *i.p.* for 3 days) or reserpine (3 mg/kg, *i.p.* for 2 days). An intraventricular injection of atropine (5–10 μ g/rat) or morphine (10 μ g/rat) exerted a facilitatory action on the spindle, while an intrathalamic and intracaudate injection of atropine (5–50 μ g/rat) showed a suppressive action. The spindle was also markedly enhanced by the microinjection of atropine (15–30 μ g/rat) or morphine (30 μ g/rat) into the reticular formation, and these actions were completely antagonized by eserine (0.3 mg/kg, *i.v.*), but only partially by methysergide (4 μ g/rat, intraventricular administration.) These results suggest that the cholinergic system may play an important role in the regulation of the caudate spindle.

Keywords—caudate spindle; atropine; morphine; microinjection; rat

INTRODUCTION

When the head of the caudate nucleus is stimulated electrically or chemically, a spindle burst (caudate spindle) is elicited in the cortex, thalamus and contralateral caudate nucleus in the cat¹⁾ and monkey.²⁾ The caudate spindle is accompanied by a change in behavioral activity. The electrical stimulation of the caudate nucleus at a low frequency makes a change in animal behaviours from an alert or active state to a resting state with drowsiness³⁾ and, moreover, the ability of the animal to pursue specific tasks, *e.g.* performing a learned activity, decreased consistently.⁴⁾ Buchwald *et al.*¹⁾ reported that the caudate spindle was inhibited by high frequency electrical stimulation of the reticular formation, while a condition of sleep facilitated the occurrence of the spindle. Furthermore, it has been reported that dopaminergic blocking agents enhanced the caudate spindle^{5,6)} whereas the neuroleptics inhibited it.⁷⁾ The caudate spindle was studied mainly in the cat, however, there has been no attempt to

study the spindle in the rat, even though it has frequently been employed as an experimental animal in the study of CNS-acting agents. Therefore, we performed a study concerned with the spindle in the rat in an attempt to examine the neural mechanism of the development of the caudate spindle.

MATERIALS AND METHODS

All experiments were carried out on male rats of Wistar-strain weighing 250–300g. The animals were anesthetized with ether, immobilized with gallamine triethiodide, ventilated artificially, placed on a stereotaxic apparatus and subsequently subjected to the surgical operation as follows. A midline incision was made and the skin and muscle of the head were reflected to expose the cranium. The locus for an electrode insertion was determined according to the stereotaxic atlas of Pellegrino and Cushman.⁸⁾ A hole was made by drilling at the portion on the cranium using a dental drill. Screw electrodes were

inserted through the cranium until their tips arrived at the dura overlying the frontal and parietal cortex. The caudate nucleus was impaled with a stainless steel concentric bipolar electrode for an electrical stimulation and simultaneously with a cannula-electrode for a microinjection of drugs. The outer sheaths of the former and latter electrodes were 0.5 and 0.8 mm in diameter, respectively. An inner stainless steel wire or tube protruded from the tip of the sheath by approximately 0.5 mm. The caudate spindle was induced by electrical stimulation of the caudate nucleus (1 ms, 0.1 Hz, 5 V) with the implanted electrode and recorded from the frontal and parietal cortex bipolarly. Forty min after the operation, measurements of the caudate spindle were started and, after another 20 min, the drug administration was initiated. In some experiments, drugs were intracerebrally injected through the cannulae electrode using a microsyringe.

An extracellular single unit recording was also utilized so as to confirm the effects of atropine on the single cell of the reticular formation. Rats were anesthetized with ether and placed on a stereotaxic apparatus as described above. The body temperature was maintained at 37°C. Glass micropipettes (1–3 megohms) filled with 2 M sodium chloride were inserted into the mesencephalic reticular formation. The potentials were passed through a high-impedance amplifier,

monitored on an oscilloscope and recorded with a rate meter.

Recording and microinjection sites were the caudate nucleus (A: 7.2, L: 3.0, H: +1.6), the anteroventralis thalamic nucleus (A: 5.4, L: 1.8, H: -0.8), and the mesencephalic reticular formation (A: 1.6, L: 2.3, H: -1.9). The site where the tips of the electrodes located was histologically examined after the end of experiment.

RESULTS

1. General Observation

Figure 1 shows electrical activity recorded in the frontal and parietal cortex when the caudate nucleus was stimulated at 0.1 Hz. The caudate spindle was clearly detected in the frontal cortex, but not in the parietal cortex. Thus, in the present experiment, the influence of various drugs was studied on the caudate spindle in the frontal cortex.

2. Influence of the Cholinergic System

In 5 out of 6 rats, the duration of the caudate spindle was significantly increased 20 min after the administration of atropine (3–10 mg/kg, *i.v.*) (Fig. 2) and became approximately twice that of the control after 30 min (Table I). On the other hand, the caudate spindle was abolished 5 min after the administration of eserine (0.3 mg/kg, *i.v.*) in all rats (data not shown).

3. Influence of Catecholaminergic and Serotonergic

FIG. 1. The Spindle Waves induced by Electrical Stimulation of the Caudate Nucleus (1 ms, 0.1 Hz, 5 V) in the Immobilized Rat

At the dot, the stimulation was given. FC: frontal cortex, PC: parietal cortex.

FIG. 2. Effect of Atropine (10 mg/kg, *i.v.*) on the Caudate Spindle

At the dot, electrical stimulation was given.

System

Even if the rat was treated with reserpine (3 mg/kg, *i.p.* for 2 days) or L-DOPA (400 mg/kg, *i.p.*), there were no changes in amplitude and duration of the spindle (Table I). Furthermore, either pretreatment with *p*-chlorophenylalanine (PCPA) (400 mg/kg, *i.p.* for 3 days), or administration of 5-hydroxytryptophan (5-HTP) (250 mg/kg, *i.p.*) showed no appreciable influence on the appearance of the caudate spindle in all rats (Table I).

4. The Mode of Action of Morphine on the Caudate Spindle

In 18 out of 20 rats, the caudate spindle was markedly increased in both amplitude and dura-

tion 30 min after the administration of morphine (3–10 mg/kg, *i.v.*) as shown in Fig. 3 and Table I, respectively. The enhancement of the caudate spindle by morphine as well as control response were completely abolished by eserine (0.3 mg/kg, *i.v.*) but not by methysergide (1 mg/kg, *i.v.*) (Fig. 4). However, pretreatment with reserpine (3 mg/kg, *i.p.* for 2 days) or PCPA (400 mg/kg, *i.p.* for 3 days) did not bring about any alterations in the effect of morphine on the caudate spindle (data not shown).

5. Effects of Microinjection of Atropine and Morphine into Various Sites of the Brain on the Caudate Spindle

In order to examine the site of action of

TABLE I. Changes in the Amplitude and Duration of Caudate Spindle following the Treatment with Various Drugs

Drugs	Dose	Route	Caudate spindle							
			Amplitude (μ V)				Duration (s)			
			Control	15min	30min	1h	Control	15min	30min	1h
Atropine	10 mg/kg	<i>i.v.</i>	80.2 $\pm$ 3.6	83.3 $\pm$ 3.3	80.4 $\pm$ 3.5	—	2.3 $\pm$ 0.2	4.0 $\pm$ 0.2 ^{c)}	4.8 $\pm$ 0.3 ^{c)}	—
Morphine	10 mg/kg	<i>i.v.</i>	81.3 $\pm$ 2.5	92.2 $\pm$ 2.6 ^{b)}	92.6 $\pm$ 3.6 ^{a)}	—	1.8 $\pm$ 0.2	4.7 $\pm$ 0.3 ^{c)}	3.8 $\pm$ 0.2 ^{c)}	—
L-DOPA	400 mg/kg	<i>i.p.</i>	92.8 $\pm$ 5.4	—	86.9 $\pm$ 4.5	86.4 $\pm$ 2.0	2.3 $\pm$ 0.1	—	2.0 $\pm$ 0.1	2.1 $\pm$ 0.1
5-HTP	250 mg/kg	<i>i.p.</i>	77.4 $\pm$ 4.3	—	73.7 $\pm$ 3.6	81.3 $\pm$ 2.5	1.4 $\pm$ 0.1	—	1.3 $\pm$ 0.1	1.8 $\pm$ 0.2
Atropine	10 μ g/rat	intoRF ^{d)}	90.0 $\pm$ 5.9	104.3 $\pm$ 2.9 ^{a)}	100.9 $\pm$ 5.4	—	1.9 $\pm$ 0.3	3.1 $\pm$ 0.2 ^{b)}	2.9 $\pm$ 0.2 ^{b)}	—
Morphine	10 μ g/rat	intoRF ^{d)}	93.0 $\pm$ 3.7	98.2 $\pm$ 4.1	98.6 $\pm$ 3.8	—	2.0 $\pm$ 0.2	3.6 $\pm$ 0.2 ^{c)}	3.4 $\pm$ 0.1 ^{c)}	—

a) $p < 0.05$, b) $p < 0.01$, c) $p < 0.001$, d) RF: reticular formation.

FIG. 3. Effect of Morphine (10 mg/kg, *i.v.*) on the Caudate Spindle
At the dot, electrical stimulation was given.

FIG. 4. Effects of Eserine and Methysergide on the Morphine-enhanced Caudate Spindle

A and C, 30 min after administration of morphine (10 mg/kg, *i.v.*); B, 15 and 45 min after administration of eserine (0.3 mg/kg, *i.v.*) and morphine, respectively; D, 25 min after administration of methysergide. At the dot, electrical stimulation was given.

atropine and morphine, microinjection of the drug into the brain was made. The administration of 5 to 50 μg of atropine into the caudate nucleus or anteroventralis thalamic nucleus suppressed the caudate spindle in 14 out of 17 rats (Fig. 5).

FIG. 5. *Effects of Microinjection of Atropine and Morphine into Various Sites of the Brain on the Caudate Spindle*

A, intracaudate injection of atropine (50 $\mu\text{g}/\text{rat}$); B, intrathalamic injection of atropine (10 $\mu\text{g}/\text{rat}$); C, intraventricular injection of atropine (10 $\mu\text{g}/\text{rat}$); D, intraventricular injection of morphine (10 $\mu\text{g}/\text{rat}$); At the dot, electrical stimulation was given.

FIG. 6. *Effects of the Microinjection of Atropine into the Reticular Formation on the Caudate Spindle*

A, control response; B, 45 min after the microinjection of atropine (10 $\mu\text{g}/\text{rat}$) into the reticular formation; C, 30 min after the administration of eserine (0.3 mg/kg, i.v.). At the dot, electrical stimulation was given. Note that the facilitatory action of atropine on the caudate spindle was antagonized by eserine.

On the other hand, intraventricular administration of atropine (5–10 $\mu\text{g}/\text{rat}$) or morphine (10 $\mu\text{g}/\text{rat}$) produced a marked potentiation of the caudate spindle. Typical examples are shown in Fig. 5. The microinjection of atropine (15–30 $\mu\text{g}/\text{rat}$) or morphine (30 $\mu\text{g}/\text{rat}$) into the reticular formation produced a significant enhancement of the caudate spindle, and these effects as well as the control response were completely abolished by eserine (0.3 mg/kg, i.v.) as shown in Fig. 6 and 7, respectively. On the other hand, when methysergide (4 $\mu\text{g}/\text{rat}$) was administered intraventricularly, morphine-induced enhancement of the spindle was abolished in amplitude, but not in duration in all rats examined.

6. Effects of Atropine and Eserine on the Single Cell in the Reticular Formation

In order to confirm the actions of atropine and eserine, an extracellular recording on a single cell in the reticular formation was carried out. In 11 out of 25 cells, the administration of atropine (10 mg/kg, i.v.) produced a long-lasting depression of activity after an initial temporal oscillation (Fig. 8). The firing rate returned to the base line level after 70–90 min. In 7 cells in which responses were examined after atropine administration (5 mg/kg, i.v.), the duration of inhibition varied over 17 and 50 min. As shown in Fig. 8, this inhibitory effect of atropine on the firing rate of the cell was antagonized by eserine (0.3 mg/kg, i.v.).

DISCUSSION

The finding in the present investigation that the stimulation of the caudate nucleus induced spindle in the rat cortex is consistent with the observation in cats¹⁾ and monkeys.²⁾ Recently, Ishikawa and Yamamoto⁹⁾ have reported that the caudate spindle in the cat was abolished by eserine and this inhibitory action of eserine was antagonized by atropine. They then suggested that a cholinergic mechanism is intimately participating in the inhibition of the caudate spindle.

In the present study, we also demonstrated that the caudate spindle in the rat was enhanced by atropine, but was suppressed by eserine.

However, reserpine, L-DOPA, PCPA and 5-HTP had no influence on the spindle, indicating that only the cholinergic mechanism is responsible for the inhibition of the spindle in the rat. The facts that morphine also enhanced the spindle and that the enhancing action of morphine was antagonized by eserine but not by methysergide, PCPA and reserpine may also support the feasibility that the cholinergic system is involved in the suppression of the caudate spindle.

In order to examine the site of action of atropine and morphine, microinjection of the drugs into the brain was performed. Such microinjection is quite advantageous in that a relatively circumscribed neuronal pool can selectively be stimulated. The microinjection of atropine into the caudate nucleus or the anterioventralis thalamic nucleus suppressed the caudate spindle. In favor with these findings, Buchwald and Hull,¹⁰⁾ Langlois and Poussart¹¹⁾ and Poussart and Langlois¹²⁾ reported that local injection of acetylcholine into the caudate nucleus or thalamic nucleus

could elicit a spindle which is quite similar to that induced by electrical stimulation. It is, therefore, likely that the site of action of atropine is the lower brain site. That the intraventricular injection of atropine produced a marked enhancement of the caudate spindle is also in agreement with this assumption.

Local administration of atropine into the reticular formation brought about a marked enhancement of the caudate spindle. Therefore, the cholinergic system in the reticular formation can be assumed to play an important role in the inhibition of the caudate spindle in the rat. This assumption is supported by the evidence that atropine did depress the firing rate of single cells in the reticular formation and that this action was completely antagonized by eserine. With respect to the regional significance of the cholinergic nervous system for the inhibition of the caudate spindle in the rat, furthermore, the reticular formation appears to be of particular importance. This view is based on the following facts. First, local admin-

FIG. 7. *Effects of the Microinjection of Morphine into the Reticular Formation on the Caudate Spindle*

A, control response; B, 30 min after the microinjection of morphine (10 µg/rat) into the reticular formation; C, 30 min after the administration of eserine (0.3 mg/kg, i.v.). At the dot, electrical stimulation was given. Note that the facilitatory action of morphine on the caudate spindle was antagonized by eserine.

FIG. 8. *Effects of i.v. Administration of Atropine and Eserine on the Spontaneous Firing Rate of a Single Cell in the Reticular Formation*

Upper tracing represents the effects of atropine (10 mg/kg, i.v.) and eserine (0.5 mg/kg, i.v.) on the firing. Lower tracing shows the effect of eserine (0.3 mg/kg, i.v.) on the atropine-suppressed response. Note that the inhibitory action of atropine on the firing response was antagonized by eserine. At the arrow, drugs were given.

istration of atropine into the reticular formation brought about a marked enhancement of the caudate spindle. Secondly, atropine did suppress the firing rate of single cells in the reticular formation and, moreover, this action of atropine was completely antagonized by eserine. Of particular interest in this connection is the observation of Ishikawa and Yamamoto⁹⁾ that the caudate spindle was inhibited by high frequency stimulation of the reticular formation and this inhibitory effect was abolished by intravenous administration of atropine.

In the present study, morphine was found to exert a potent facilitatory action on the caudate spindle. Although the mode of action of morphine may be complicated, this facilitation is conceivably attributable to the inhibitory action of morphine on ACh release,^{13,14)} because the facilitation of the spindle by morphine was completely abolished by eserine, only partially by methysergide given intraventricularly, but not by reserpine and PCPA. Ishikawa and Yamamoto⁹⁾ suggested that a serotonergic system may also be related to the inhibition of the caudate spindle. In the present study, methysergide did not show any effect when given intraventricularly but did show a partial suppressive effect on morphine enhanced facilitation of the spindle when given intraventricularly. Therefore, the possibility can not be excluded that the serotonergic system is also involved in the manifestation of the morphine enhanced caudate spindle in the rat. With respect to the evaluation of the serotonergic mechanism on the caudate spindle, however, further investigation is required.

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