EFFECTS OF 1-METHYL-5-CHLOROINDOLINE METHYLBROMIDE (S-6) ON RENSHAW CELLS OF CATS

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Abstract—Effects of 1-methyl-5-chloroindoline methylbromide (S-6) on Renshaw cells were investigated in cats anesthetized with pentobarbital sodium. S-6 proved to have little effect on spike discharges of Renshaw cells when administered intravenously, but the agent electrophoretically applied revealed excitatory effects which resembled the action of methacholine and such effects were blocked by intravenous administration of atropine. It was concluded that (1) S-6 is impermeant to the blood brain barrier and (2) the excitatory action of S-6 on Renshaw cells is muscarinic in nature.

Previous reports from this laboratory have demonstrated that 1-methyl-5-chloroindoline methylbromide (S-6) exerts a remarkable parasympathomimetic effect on various peripheral organs (1, 2), and that i.v. administration revealed little effect on activities of the central nervous system, such as EEG, spinal reflexes and anti-convulsive activity (3). These results suggested that intravenously administered S-6 may not enter the central nervous system.

In the present study, the effects of electrophoretically administered S-6 on Renshaw cells were compared with the results seen with intravenous administration. The action of electrophoretically applied S-6 on the electrical activity of the Renshaw cells was also compared with that of other cholinergic agents.

MATERIALS AND METHODS

Experiments were carried out on 29 adult cats anesthetized with pentobarbital sodium (35 mg/kg i.p.) and immobilized with gallamine triethiodide. The head and pelvis of the animal were stereotaxically fixed.

Laminectomy was performed at levels from L1 to S2. The L7 ventral root was sectioned peripherally and the central end was placed on a bipolar platinum wire electrode for stimulation by square pulses of 0.1 msec duration at a rate of 1 Hz.

Double or triple barrel microelectrodes were used for electrophoretic application of S-6 and other drugs to Renshaw cells. Unitary activities of Renshaw cells were recorded extracellularly with one barrel filled with 4 M NaCl (tip resistance, 1–3 MΩ). The other barrels were filled with one or two of the following drugs dissolved in 0.9% saline: S-6 (2 M, pH 3.9), methacholine bromide (Tokyo Kasei) (2 M, pH 3.2), acetylcholine chloride (Daiichi Seiyaku) (1 M, pH 4.1) and nicotine tartrate (Nakarai Kagaku Yakuhin) (1 M, pH 2.9). The tip resistances of drug-containing barrels were kept below 20 MΩ to avoid
resistance change during passage of current. Negative retaining current (10-25 nA) was applied to each of the barrels to prevent outward diffusion of the drugs from the tip. The drugs were ejected by 50-100 nA positive current applied to each barrel through a resistor of 100 MΩ and a platinum wire. The amount of applied current was monitored on an oscilloscope.

The unitary activity of Renshaw cells was fed to a high input-impedance preamplifier and displayed on a dual-beam oscilloscope in order to take photographs in a conventional manner. Mean frequencies of both spontaneous and induced discharges of Renshaw cells in every 100 msec were recorded on a tachograph, as the number of spikes per second.

The body temperature was maintained at about 37°C by a heating pad and radiant heat. The exposed spinal cord was covered with mineral oil kept at about 37°C.

The substance S-6, water-soluble white crystalline powder (Fig. 1) was synthesized in the Kyorin Chemical Laboratory.

RESULTS

Identification of Renshaw cells

Forty-one cells were used for the present study and were located ventromedially in the ventral horn of the lumbar cord. Such were identified as Renshaw cells on a basis of the following criteria adopted by Renshaw, Eccles et al. and Curtis et al. (5-9).

1) A high frequency train of 7-17 spikes was evoked by a volley in the ventral root. The first spike appeared with a latency of 0.7-1.7 msec, and the firing frequency which was initially as high as 800 Hz gradually fell over a period of 10-45 msec; 2) spontaneous discharges at rates of 5-10 Hz; 3) activation by electrophoretic application of acetylcholine (see Fig. 4).

Effects of S-6 and other cholinergic drugs on Renshaw cells

Effect of S-6: Little effect of S-6 was detected on the spike discharges by Renshaw cells when the drug was administered i.v. even at a dose sufficient to produce cholinomimetic effects on the various peripheral organs (5 mg/kg) (1-3). In contrast, the spike discharges of Renshaw cells were affected by S-6 when it was released locally, either by removing the negative retaining current or by applying positive ejecting current to S-6-containing electrodes.

Fig. 2 shows an example of firing pattern of Renshaw cells (20 cells tested) and the effect of electrophoretically applied S-6. Application of positive current (100 nA) to S-6-containing barrel induced bursts of spike discharges. Each of the bursts lasted for a short period and consisted of 10-17 spikes firing at rates of 500-600 Hz (Fig. 2B). Such bursts were never observed in spontaneous discharge of Renshaw cells under our experimental conditions (Fig. 2A). Double or triple spikes persisted after cessation of the application.
Fig. 2. Typical spontaneous firing patterns of a Renshaw cell and the effect of electrophoretic administration of S-6. A: spontaneous discharges. B: burst-like activities induced by application of 100 nA positive current to a barrel containing 2 M S-6. C, D and E: recovery phases at 0, 15 and 30 sec after cessation of the current application, respectively. Calibrations: vertical: 200 μV, negative downwards; time base: 100 msec for all records. The result shown is typical of 20 cells tested.

Fig. 3. Time course of change in discharge frequency of Renshaw cells (typical of 15 cells tested) produced by electrophoretic administration of S-6, methacholine, acetylcholine and nicotine. Abscissae: time scale = 5 sec/div. Ordinates: mean discharge frequency in every 100 msec (spikes per second). Horizontal bars represent periods of electrophoretic application of 2 M S-6 (A), 2 M methacholine (B), 1 M acetylcholine (C) and 1 M nicotine (D). Positive current of 100 nA was applied for A and B, and that of 50 nA was applied for C and D.

FIG. 2. Typical spontaneous firing patterns of a Renshaw cell and the effect of electrophoretic administration of S-6. A: spontaneous discharges. B: burst-like activities induced by application of 100 nA positive current to a barrel containing 2 M S-6. C, D and E: recovery phases at 0, 15 and 30 sec after cessation of the current application, respectively. Calibrations: vertical: 200 μV, negative downwards; time base: 100 msec for all records. The result shown is typical of 20 cells tested.

Fig. 3 illustrates the time course of the mean frequency of spontaneous and S-6-induced discharges observed with the same cell as that shown in Fig. 2. The firing rate before the application of current was 3–7 Hz. The discharge frequency was gradually increased by the current application after a period of a depression for 10–15 sec and reached a plateau of 15–20 Hz. The maximum frequency of 50–60 Hz was attained immediately after the termination of the current application. The frequency then gradually decreased, reaching the control level within 60–120 sec. The longer the period of the current application the more prolonged was the after-effect.

Thus, the burst-like spike discharges (Fig. 2) and the slow excitation accompanied by the preceding depression (Fig. 3A) appeared characteristic of the actions on Renshaw cells of locally applied S-6.

Effects of acetylcholine, nicotine and methacholine: It has been reported that electrophoretic application of these cholinergic drugs exerts excitatory effects on Renshaw cells.
The effects of each of the drugs differed from one another in both latency and the rate of increase in discharge frequency as well as in firing patterns (11, 12). The effects of these drugs were compared with those of S-6 in the same Renshaw cells.

Fig. 3C shows the response typical of 15 Renshaw cells tested to acetylcholine which was applied electrophoretically during the period indicated by the horizontal bar (50 nA). The increase in firing frequency began 200-300 msec after the onset of the current application and reached the maximum of 125 Hz within 3 sec. After termination of the current application, the frequency returned to the control level within 2 sec. Single spike discharges were predominantly observed during and after the current application, although double spike discharges were occasionally observed (Fig. 4).

![Fig. 4](image)

**Fig. 4.** Firing pattern of a Renshaw cell produced by electrophoretic administration of acetylcholine. Positive current (50 nA) was applied to a barrel containing acetylcholine (1 M) during the period indicated by the two triangles. All the records are continuous from top to bottom. Calibrations: vertical; 200 μV (negative downwards); horizontal; time scale=100 msec/div.

Fig. 3D shows an example of the responses of 5 Renshaw cells tested with electrophoretically applied nicotine (50 nA). The firing frequency began to increase slowly about 5 sec after the onset of the current application and was followed by a rapid increase. After termination of the current, discharge frequency continued to increase to a peak of 120 Hz, and then gradually returned to the control level within 40 sec. Single spike discharges only were observed during and after the application of nicotine.

Fig. 3B illustrates the typical response of Renshaw cells (10 cells tested) to methacholine released electrophoretically (100 nA). The rate of increase of firing frequency by methacholine was slower than that by acetylcholine or nicotine. During the current application, firing frequency increased to 20-30 Hz. The firing frequency reached a peak of 60 Hz immediately after the termination of the current and then returned to the control level within 60-120 sec. High frequency bursts of the spikes were observed during the current application. Thus, the effects of methacholine on Renshaw cells resembled those of S-6.

**Effects of atropine on the excitatory actions of S-6 and other cholinomimetics on Renshaw cells**

Intravenous administration of atropine (0.3-2.0 mg/kg) had little effect on the repetitive
discharges of Renshaw cells evoked by a volley in the ventral root but induced a reduction of their spontaneous discharges (6 cells).

Intravenous administration of atropine (0.3–1.0 mg/kg) abolished the excitatory effect of electrophoretically applied S-6 and methacholine on 6 Renshaw cells tested. An example of the abolition is shown in Fig. 5A-D. The excitation by acetylcholine, on the other hand, was only depressed by less than 20% with any of 3 cells tested (Fig. 5E-F). The excitation induced by nicotine was not affected by atropine (2 cells).

**DISCUSSION**

Pharmacological properties of Renshaw cells have been described by many investigators (7–13). It has been reported that intravenous administration of methacholine, carbamylcholine and succinylcholine had little effect on Renshaw cells, but that the cells were activated when these agents were administered electrophoretically (10, 13). This suggests that the compound which has a quaternary ammonium base does not pass through the blood brain barrier. Systemic administration of S-6, a quaternary ammonium compound of an indoline derivative (Fig. 1), exerted a remarkable parasympathomimetic effect on various peripheral organs (1, 2). Our previous studies on the distribution of systemically administered H³-S-6 (4) as well as its effects on the central nervous system (3) suggested that S-6 does not enter the central nervous system. Such was confirmed in the present study, in which S-6 was not effective when administered intravenously, but was effective when applied electrophoretically to Renshaw cells.
The excitatory action of S-6 revealed a slow onset and recovery of the firing rate in contrast to the rapid action of acetylcholine. It has been reported that the action of the latter compound was due mainly to interaction with nicotinic receptors which had a faster onset and recovery than that due to interaction with muscarinic receptors (12). In this regard, S-6 seems to interact with the muscarinic receptor.

Excitatory effects of electrophoretically applied S-6 on Renshaw cells could be divided into two stages; the early excitation preceded by a brief period of depression during the current application and the late excitation after cessation of the current application. A similar pattern of firing of Renshaw cells has been reported by Curtis and Ryall (11) with application of methacholine. These authors demonstrated that the frequency in the early excitatory phase failed to rise above a plateau despite increase in applied current whereas that in the late phase was increased with increasing current intensity. It was found, in the present study, that application of 100 nA positive current to NaCl-containing electrodes instantaneously depressed spontaneous firing of Renshaw cells, and that a similar depression also appeared by application of the same amount of positive current to S-6-containing electrodes. These findings suggest that the brief depression of the firing during the application of S-6 is due mainly to the direct action of the positive current on Renshaw cells. Desensitization of these cells produced by this compound, however, might partly be responsible for the depression as proposed by Curtis and Ryall (11).

High frequency spike bursts were found to be characteristic of the response of Renshaw cells to local application of S-6. Such a burst activity was never observed in spontaneous discharges in the present study, although it has been reported that the burst-like spontaneous firing of Renshaw cells was occasionally observed. Such burst-like discharges of Renshaw cells were depressed by atropine but resistant to dihydro-β-erythroidine (9). Furthermore, the burst activities were reportedly induced by the application of acetylcholine even in the presence of dihydro-β-erythroidine (12) as well as by application of dl-muscarnine and methacholine (11). Therefore, the bursts seem to be related to interaction of drugs with muscarinic receptors of Renshaw cells. This view is also supported by our results that high frequency spike bursts could not be induced by application of nicotine. In addition, antagonistic effects of atropine on S-6-induced excitation of Renshaw cells indicate that this excitatory action was induced by muscarinic action of this compound.

Prolongation of the depression of Renshaw cells during the application of S-6 and methacholine after administration of atropine is ascribed to reduction in the spontaneous firing rate caused by atropine per se.

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