PHARMACOLOGICAL ACTIONS OF INTRACEREBRALLY ADMINISTERED POLYAMINES IN MICE*

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Accepted January 31, 1977

Abstract—Pharmacological actions of spermidine (SPD) and spermine (SPM) in mice given these polyamines by intracerebral injection (i.c.) were investigated. The spontaneous motor activity (SMA) assessed by the photocell counter method did not change immediately following injection of 80 μg SPD, but was enhanced 24 hours after the injection of 40 or 80 μg. A significant decrease of SMA was not immediately evident after the administration of 40 μg SPM but was demonstrated 24 hours later. Mice given single doses of 20 μg SPD exhibited increase in weight gain and those given 40 μg showed no significant difference, whereas a conspicuous decrease in weight occurred in mice given 80 μg of SPD. The body weights of mice given single doses of 10 μg SPM remained much the same, while significant weight losses occurred in both the SPM 20 μg and 40 μg dosed groups. There was a significant prolongation of pentobarbital induced sleep 6 days after the injection of high doses of SPD or SPM. Similar prolongation of sleeping time was also evident 30 minutes after the injection of SPM. A maximal hypothermic response with a fall by about 1°C was observed immediately following injection of 80 μg of SPD and 24 hours after the injection of 40 μg SPM, respectively.

Detailed studies from the wide-spread distribution of the polyamines, spermidine (SPD) and spermine (SPM) in various tissues (1, 2) to the central nervous system in the rat, rabbit, dog, monkey and human have been reported (3–7), however, the physiological role of these polyamines in brain tissues remains unknown.

We previously reported the pharmacological effects of i.p. administered SPD and SPM (8), but the question remains whether all those actions are mediated by the central nervous system. The present study was an attempt to assess the pharmacological actions of these polyamines in mice when the amines were injected directly into the brain.

MATERIALS AND METHODS

Materials

Male ddY mice weighing 25 to 30 gm were used. Drugs used were spermidine phosphate (Tokyo Kasei), spermine phosphate (Tokyo Kasei) and pentobarbital sodium (Abbott). The technique employed for i.c. administration was that described by Brittain and Handley (9) and a modification of the procedure of Haley and McCormick (10). The drugs were

* Part of the data herein was reported at the 27th (October, 1976) Kita Regional Meeting of the Japanese Pharmacological Society
dissolved in physiological saline and were given to the animals in a volume of 0.02 ml per animal i.c.. Groups of ten or twelve mice were housed in individual 275 x 165 x 135 cm rectangular cages with free access to food and water. All experiments were done between 2:00 p.m. and 7:00 p.m. Each animal was used only once.

**Measurement of SMA**

The effects of drugs on SMA were measured by the photocell counter method. A pair of mice was placed in a small, soundproof dark-box (20 x 20 x 20 cm) that had a single photocell light so that the frequency of spontaneous movement, i.e. moving across the light beam, of the animals could be counted automatically. The test was performed after a 15 min acclimatization period.

**Measurement of body temperature**

Effects of SPD and SPM on normal body temperature were studied in groups of ten mice with temperatures of 37 – 38°C. The rectal temperature of each animal was measured with a thermister-type thermometer (Nåtsume) at a room temperature of 25°C.

**Effects of SPD and SPM on pentobarbital sodium induced sleep**

Animals were given SPD or SPM 30 min or 6 days before the i.p. injection of pentobarbital sodium (50 mg/kg) in order to assess the effects of the polyamines on sleeping time. Sleeping time was considered as the duration of complete disappearance of right reflex.

**RESULTS**

*SMA following i.c. injection of SPD and SPM*

Fig. 1 includes the results of the SMA by the photocell counter method in mice during the 90-minute period immediately, 24, 48 and 144 hr after the injection in doses of 20, 40 and 80 μg SPD. A significant increase of SMA was found from 24 hr after the injection of 40 or 80 μg SPD, whereas during the 90-minute period immediately following injection, there was no significant change. Namely, the group given 40 μg SPD showed an average

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**Fig. 1.** Effect of an intracerebral injection of spermidine on spontaneous motor activity measured by the photocell counter method. *: p < 0.01 when compared with saline treated group. **: p < 0.05 when compared with saline treated group. : saline, : 20 μg, : 40 μg, : 80 μg. Results are the mean and standard error of 12 animals per group.
Effect of an intracerebral injection of spermine on spontaneous motor activity measured by the photocell counter method. **: p < 0.05 when compared with saline treated group. ■: saline, □: 20 μg, △: 40 μg, ▼: 80 μg. Results are the mean and standard error of 12 animals per group.

Count of 179 and the group given 80 μg SPD a mean count of 476, respectively, in contrast to an average count of 80 μg for the control group on saline. The SPD 80 μg dosed group continued to exhibit a significantly increased SMA 48 hr after the injection, but at 144 hr post injection, there was a significant decrease in the SMA of these animals.

Data in Fig. 2 are results of the same tests on mice given 10-40 μg SPM. Immediately after the injection, the SPM 40 μg dosed group did not display a significant decrease in SMA. The SPM 40 μg dosed group showed a significant decline 24 hr after the injection; the average count with this SPM-treated group was 13 as against 80 for the saline-treated control group. However, the average count at 48 and 144 hr after the injection did not differ significantly between the drug treated group and the controls.

Body weight changes following administration of SPD or SPM

Body weight changes after the administration of SPD in doses of 20-80 μg are shown in Fig. 3. The groups given 20 μg of SPD displayed significantly greater, marked increases from 2 days after the injection and up to the sixth day as compared with the saline-treated control group. The SPD 40 μg dosed group showed essentially the same pattern of weight gain as the saline controls throughout the 6-day period of observation, while the body weight began declining on the first day after the administration of 80 μg SPD and fell substantially thereafter to the sixth day.

The corresponding data obtained from mice injected with 10-40 μg of SPM are presented in Fig. 4. The pattern of weight gain observed with the group dosed with 10 μg of SPM did not differ from that with the saline-treated control group, whereas with the groups given 20-40 μg of SPM, the body weight began to decline 1 day after the injection, reached a peak of weight loss on the second day and subsequently showed a trend to recovery on the fifth or sixth days.

Body temperature after the i.c. injection of SPD or SPM

Body temperature changes following administration of SPD or SPM are shown in Figs. 5 and 6. The average body temperature did not differ significantly between the group dosed
FIG. 3. Changes in body weight produced by an intracerebral injection of spermidine. 
*: p<0.01 when compared with saline treated group.  ---: saline,  • : 20 µg,  △: 40 µg,  ⊙ : 80 µg. Results are the mean and standard error of 12 animals per group.

FIG. 4. Changes in body weight produced by an intracerebral injection of spermine. 
*: p<0.01 when compared with saline treated group.  ---: saline,  • : 10 µg,  △: 20 µg,  ⊙ : 40 µg. Results are the mean and standard error of 12 animals per group.
with 40 μg SPD and the saline-treated control group except for a significant fall 1 day after
the administration. In the group given 80 μg of SPD, on the other hand, the temperature
began to fall 15 minutes after the injection and reached a peak at 45 minutes, followed by a
tendency toward recovery. However, this group subsequently displayed a significant
redeline on the third and fourth days.

Immediately following injection with 20 μg of SPM, a slight fall in temperature occurred
although normal values were soon restored. The temperature fell again 1 day after the
injection, and returned to the control level 4 days post injection. A similar tendency, yet
with more marked changes, was observed after 40 μg SPM; the rectal temperature of
animals in this group lowered by approximately 1 °C as compared with the control 1 day
after the injection.

Influence of the administration of SPD or SPM on pentobarbital induced sleep

The data obtained as to durations of sleep induced by pentobarbital sodium (50 mg/kg)
in mice 30 minutes and 6 days after the injection of SPD or SPM are summarized in Table 1.
The mice showed no significant prolongation of the duration of pentobarbital sleep induced
30 min after the injection with SPD at any of the three dose levels, 20, 40 or 80 μg. The
duration of sleep induced 6 days after 80 μg SPD, however, was significantly prolonged.
All three groups of mice given 10, 20 or 40 μg of SPM exhibited significant prolongations of
TABLE 1. Effect of intracerebral injections of spermidine and spermine on pentobarbital induced sleeping time

| Agent    | Dose (μg) | Sleeping time (min) 30 min after i.c. injection | Sleeping time (min) 6 days after i.c. injection |
|----------|-----------|-----------------------------------------------|---------------------------------------------|
| Saline   | 0.02 ml/mouse | 37.8 ± 3.2 | 41.5 ± 3.4 |
| Spermidine | 20 | 40.6 ± 4.5 | 33.1 ± 3.8 |
|          | 40 | 47.6 ± 2.5 | 35.8 ± 3.2 |
|          | 80 | 48.5 ± 5.0 | 61.3 ± 14.9* |
| Spermine | 10 | 52.3 ± 2.6* | 31.9 ± 4.3 |
|          | 20 | 51.6 ± 2.7* | 46.7 ± 3.5 |
|          | 40 | 50.0 ± 2.5** | 61.1 ± 4.2* |

*: p < 0.01 when compared with the saline treated group.
**: p < 0.05 when compared with the saline treated group.

Results are the mean and standard error of 10 animals per group.

sleep induced by pentobarbital 30 min after the injection. This effect was still demonstrable on the sixth day after the injection of 40 μg SPM.

**DISCUSSION**

Our previous studies (8) demonstrated depression of SMA, enhancement of the hypnotic effect of pentobarbital, inhibition of writhing response induced by acetic acid, and anti-strychnine effects of the i.p. injection with SPD or SPM upon mice. Although it has been reported that neither SPD or SPM crosses the blood-brain barrier and enter the central nervous system (12), our previous findings suggested the possibility that high doses of the polyamines injected by a peripheral route might pass through the blood-brain barrier. Thus, the question arose as to whether or not the effects observed after the peripheral injection of the polyamines might be totally mediated by the central nervous system.

With a high dose of SPD, 80 μg, the SMA was found to be increased 1 day after the injection and, conversely, depressed on the sixth day post injection. About 20% of the mice given a dose of 80 μg died, and in about 30% of all these mice, an erection was induced. The depression of SMA observed immediately and 1 day after 20 μg or 40 μg of SPM is analogous to the effect produced by peripheral injected polyamines. The group of mice given a dose of 40 μg SPM also showed a mortality rate of about 10%, but did not exhibit such states of excitement as increased SMA, or the erection that occurred in animals given 80 μg of SPD; there are slight discrepancies in the effects of injection between SPD and SPM. Both the SPD 80 μg dosed group and the SPM 40 μg dosed group, they are emaciated or moribund, displayed clonic convulsions. The SMA assessed by the wheel cage method did not change to any significant extent with the administration of SPD or SPM (unpublished observation), thus the finding is different from that observed with the photocell counter test.

The group given 20 μg of SPD showed a significant greater weight gain as compared with the saline-treated control group. The weight gain in animals given 40 μg of SPD did not differ from the control while the body weight of mice in the high-dose (80 μg) group markedly decreased. These results indicate that polyamines injected in low doses bring
forth promotion of growth while depression of weight gain occurs when these amines are administered at high dose levels. The concentration of polyamines was observed to be higher in the young than in adult animals (2, 11). The growth promoting effect of administered polyamines in low doses appears to be in accord with a possible interrelationship between the cerebral polyamine levels and growth. It has been reported (13) that SPD and SPM produced anorexia and adipsia in mice. It seems likely that weight loss produced by the anorexia and adipsia would be caused by a direct action on the satiety or the feeding center in the hypothalamus.

In addition, the experiments have demonstrated that these polyamines are long-acting agents. The pharmacological properties were evident in their actions of lowering body temperature and of enhancing the hypnotic effect of pentobarbital. In animals given 40 μg SPM, the body temperature fell with a maximum decrease of only about 1 °C 24 hr after the injection and the hypothermal response lasted for 3 days. This observation is not in line with respect of the degree of effect, with data reported by Anderson et al. (13) who found a maximal decrease by more than 2 °C. The discrepancy seems to be due to the difference in salt form of the polyamines in the experiments, i.e. hydrochlorides vs. phosphates. A similar discrepancy was observed with the polyamines injected by the i.p. route. There was a significant prolongation of the duration of pentobarbital-induced sleep following i.p. administration of the polyamines. The result was similar to that obtained by the i.p. injection of polyamines (4), although the enhancing effects were somewhat more pronounced in the case of the i.c. injection.

The first phase of action produced by SPD or SPM, i.e. hypothermia, the potentiation of pentobarbital and changes in SMA, may reflect the direct action of SPD or SPM in the brain. The secondary phase of action may be attributed to necrotic lesions in the brain as pathological findings were reported (13) or the slow process in a half-life 3H-labelled spermidine after intracisternal injection of 3H-putrescine in the rat as Russell et al. demonstrated (14). The results indicate that these polyamines injected i.c. produce essentially the same effects as those observed after their i.p. administration and are substances which exert sustained inhibitory actions on the central nervous system. The underlying mechanism of actions are yet to be demonstrated by detailed electrophysiological and histological studies.

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