EFFECTS OF POLYAMINES ON THE CENTRAL NERVOUS SYSTEM*

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Abstract—Effects of polyamines on the central nervous system were studied in mice. Intraperitoneal administration of Spermidine (SPD) and Spermine (SPM) decreased spontaneous motor activity as measured by either the photo-cell counters method or the open-field test and lowered rectal temperature. A significant prolongation of sleeping time after pentobarbital was confirmed in small doses of SPD and SPM which had slight influence on spontaneous motor activity. The time to convulsion and death induced by strychnine was elongated by SPD and SPM. SPM in small doses inhibited writhing responses induced by 0.7\% acetic acid. In addition, methamphetamine-induced hyperactivity and conditioned avoidance response were blocked by SPM in doses which decreased spontaneous motor activity. In all experiments, SPM appeared to have a powerful pharmacological activity compared with SPD. LD50 for SPD and SPM was 620 (500–769) mg/kg i.p. and 310 (200–480) mg/kg i.p., respectively.

The polyamines SPD and SPM and their precursor, putrescine are known to be distributed widely in animal tissues (1–3). The distribution, developmental alteration, metabolism and turnover of the polyamines in different regions of brain have recently been described (4–6). We also found that brain SPD and SPM levels were changed by long-term isolation and olfactory bulb lesions in mice (7–9). There is, however, little information concerning polyamine related pharmacological studies on the central nervous system, though effects of the polyamines on the peripheral nervous system have been discussed (10–14).

The purpose of the present paper was to obtain a central pharmacological spectrum of the polyamines, SPD and SPM, administered intraperitoneally.

MATERIALS AND METHODS

Male mice (ddY-strain) weighing 18–25 g were injected i.p. with the solutions of SPD phosphate, SPM phosphate, methamphetamine hydrochloride and sodium pentobarbital prepared with physiological saline in a dose 0.1 ml/10 g body weight and the following methods were employed:

Spontaneous motor activity (SMA)

Both the photo-cell counters method and the open-field test were employed for the measurements of SMA.

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In the photo-cell counters method, pairs of mice were placed in the sound-proof and dark cage (15 x 15 x 20 cm) which was fitted with a light beam and photoelectric cell. For purposes of familiarization all pairs of mice were placed in the test cage for 15 min before administration of SPD or SPM. Counting for a group was continued for 2 hr after administration of SPD or SPM.

In the Hall's open-field apparatus (15), ambulation (the number of blocks traversed in the open-field apparatus) as parameters of SMA was measured during a 1 min period, 30 min after administration of SPD or SPM.

Anti-methamphetamine test

Methamphetamine (5 mg/kg i.p.) was administered 30 min after SPM. The effect of SPM on hyperactivity induced by methamphetamine was measured by the photo-cell counters method.

Motor incoordination

Rotating rod was employed for the measurement of motor incoordination. The apparatus and the method were similar to those described by Dunham and Miya (16). A wooden rod with a diameter of 4 cm was rotated at a speed of 12 r.p.m. Only the mice which stayed on the rotating rod for 1 min in two successive trials were used.

Potentiation of hypnotic action of pentobarbital

Groups of 10 mice were given SPD or SPM and 30 min later 50 mg/kg of sodium pentobarbital was injected i.p.. Duration of loss of the righting reflex was measured. The potentiation of hypnotic action of pentobarbital was determined to be positive when sleeping time in drug-treated mice was prolonged more than twice that in saline treated mice.

Writhing induced by 0.7% acetic acid

Male mice in groups of 10 were given SPD or SPM intraperitoneally, 30 min before i.p. injection of 0.7% acetic acid. The number of writhings per mouse was recorded for a period of 10 min, 5 min after administration of acetic acid.

Anti-strychnine test

Groups of 10 mice were given SPD or SPM 30 min before strychnine (2 mg/kg i.p.). Two end-points were taken: the first appearance of convulsion and death.

Hypothermia

Groups of 10 mice with a rectal temperature of 37-38°C were given SPD or SPM and rectal temperature was recorded every 20 min for 2 hr at a room temperature of 25°C. A thermister probe was inserted 2.0 cm into the rectum of the animals.

Conditioned avoidance test

The pole-climbing method was employed. Details of training were as described in previous reports (17, 18). Conditioned male mice in groups of 10 were exposed to test trials after i.p. administration of SPM.
Determination of brain SPD and SPM

The separation procedure of brain polyamines was performed according to the method Endo and Ogura (19). SPD and SPM were assayed by TNBS-reaction according to the method of Satake et al. (20).

Acute toxicity

Male mice were used and intraperitoneal LD50 was determined. Mortality was recorded within 1 week after administration. LD50 was calculated by the method of Litchfield-Wilcoxon (21).

RESULTS

Spontaneous motor activity

In the photo-cell counters method, 80 mg/kg of SPD and 10 mg/kg of SPM had no effect on SMA, but more than 160 mg/kg of SPD and 80 mg/kg of SPM produced a significant decrease in SMA. ED50 for SPD and SPM was 180 mg/kg and 54 mg/kg, respectively (Fig. 1, Fig. 2, Table 6). In the open-field test, ambulation in groups injected with 80 mg/kg of SPD and 40 mg/kg of SPM was unchanged, but more than 160 mg/kg of SPD and 80 mg/kg of SPM decreased SMA significantly. ED50 for SPD and SPM was 200 mg/kg and 88 mg/kg, respectively (Fig. 3, Table 6).

Fig. 1. Effect of spermidine on spontaneous motor activity measured by the photo-cell counters method in mice. *: p<0.01 when compared with saline treated group. **: p<0.05 when compared with saline treated group. Results are the mean and standard error of 10 animals per group.

Fig. 2. Effect of spermine on spontaneous motor activity measured by the photo-cell counters method in mice. *: p<0.01 when compared with saline treated group. Results are the mean and standard error of 10 animals per group.
Fig. 3. Effect of the polyamines on ambulatory activity measured by the open-field apparatus in mice. *: p<0.01 when compared with the saline treated group. Results are the mean and standard error of 10 animals per group.

Anti-methamphetamine test

50 mg/kg of SPM did not significantly decrease the hyperactivity induced by methamphetamine, but more than 100 mg/kg produced an antagonistic effect. ED50 for SPM in the anti-methamphetamine test was 81 mg/kg (Fig. 4, Table 6).

Motor incoordination

In the rotating rod test, 50% of mice experienced motor incoordination with 500 mg/kg of SPD, 200 mg/kg and 300 mg/kg of SPM. Ninety percent of all mice were not able to stand on the rod after a dose of 700 mg/kg of SPD. However, three of 10 mice given 700 mg/kg of SPD died 2 days after administration. ED50 for SPD and SPM was 420 mg/kg and 210 mg/kg, respectively (Fig. 5, Table 6).

Potentiation of hypnotic action of pentobarbital

The polyamines produced a marked elongation of sleeping time after pentobarbital. More than 25 mg/kg of SPD potentiated significantly the sleeping time. The potentiation by SPM was more powerful than that of SPD. ED50 for SPD and SPM was 50 mg/kg and
**PHARMACOLOGY OF POLYMINES**

**Figure 5.** Effect of the polyamines in rotating rod test in mice. 10 animals per group.

**Table 1.** Effect of the polyamines on pentobarbital sleeping time in mice

| Agent   | Dose          | Sleeping time Mean ± S.E. (min) | Incidence of potentiation |
|---------|---------------|---------------------------------|--------------------------|
| Saline  | 0.1 ml/10 g   | 31.8 ± 0.9                      |                          |
| Spermidine | 25 mg/kg   | 62.8 ± 11.8**                  | 3/10                     |
|          | 50 mg/kg     | 80.4 ± 13.1*                   | 5/10                     |
|          | 100 mg/kg    | 82.7 ± 9.7*                    | 7/10                     |
| Spermine | 6.25 mg/kg   | 43.4 ± 8.9                     | 3/10                     |
|          | 12.5 mg/kg   | 66.4 ± 9.9*                    | 6/10                     |
|          | 20.5 mg/kg   | 80.4 ± 17.3*                   | 9/10                     |

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

**Table 2.** Effect of the polyamines on writhing response induced by 0.7% acetic acid in mice

| Agent   | Dose          | Number of writhings Mean ± S.E. | Inhibition (C%) |
|---------|---------------|---------------------------------|-----------------|
| Saline  | 0.1 ml/10 g   | 41.6 ± 2.3                      |                 |
| Spermidine | 50 mg/kg   | 30.6 ± 1.9*                    | 26.4            |
|          | 100 mg/kg    | 26.8 ± 3.5*                    | 35.6            |
|          | 150 mg/kg    | 14.7 ± 2.5*                    | 72.0            |
| Spermine | 12.5 mg/kg   | 29.3 ± 4.1*                    | 29.4            |
|          | 25 mg/kg     | 15.2 ± 3.1*                    | 63.4            |
|          | 50 mg/kg     | 3.5 ± 1.6*                     | 91.6            |

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

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

10 mg/kg, respectively (Table 1, Table 6).

**Writhing syndrome test**

More than 50 mg/kg of SPD inhibited significantly the writhing response induced by 0.7% acetic acid. SPM also showed the same effect in relatively small doses. ED50 for SPD
and SPM was 115 mg/kg and 16 mg/kg, respectively (Table 2, Table 6).

**Hypothermia**

A significant lowering of rectal temperature was observed 20 min after 200 mg/kg of SPD and more than 50 mg/kg of SPM. However, the temperature returned to a normal level 120 min after administration except for a large dose of SPM (300 mg/kg) (Fig. 6, Table 6).

**Conditioned avoidance test**

40 mg/kg of SPM produced a slight blocking action of conditioned avoidance response. Maximal blocking effect on conditioned avoidance and escape response was produced 30 min after 80 and 120 mg/kg of SPM. However, these effects disappeared 120 min after SPM (Fig. 7, Table 6).

**Anti-strychnine test**

SPD in a dose of 300 mg/kg and SPM in a dose of 200 mg/kg significantly elongated the time to convulsion and death induced by strychnine (Table 3, Table 6).

**Changes in brain polyamines following i.p. administrations of SPD and SPM**

Brain SPD and SPM contents in the non-treated group were 91.52±4.33 nmole/g and 104.04±4.41 nmole/g, respectively. Brain SPD and SPM contents in mice administered 320 mg/kg of SPD were 112.09±10.70 nmole/g and 145.45±7.71 nmole/g, respect-

![Figure 6](image)

**Fig. 6.** Effect of the polyamines on rectal temperature in mice. *: 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. 10 animals per group.

![Figure 7](image)

**Fig. 7.** Effect of spermine on conditioned avoidance response by the pole climbing method in mice. 10 animals per group.
TABLE 3. Effect of the polyamines on convulsion and death induced by strychnine in mice

| Agent    | Dose        | Time to convulsion (sec) | Time to death (sec) |
|----------|-------------|--------------------------|---------------------|
| Saline   | 0.1 ml:10 g | 238.6±13.45              | 318.7±21.20         |
| Spermidine | 200 mg kg  | 290.8±31.32              | 362.1±41.96         |
|          | 300 mg kg  | 310.1±25.12*             | 430.0±58.27*        |
|          | 450 mg kg  | 318.5±17.94*             | 496.2±59.92*        |
| Spermine | 50 mg kg   | 244.3±18.75              | 296.4±26.98         |
|          | 100 mg kg  | 250.0±29.20              | 352.2±33.66         |
|          | 200 mg kg  | 319.6±25.23*             | 437.4±20.70*        |

*: p < 0.01 when compared with the saline treated group.
Results are the mean and standard error of 10 animals per group.

Table 3: Effect of the polyamines on convulsion and death induced by strychnine in mice

Table 4: Brain spermidine and spermine contents 1 hr after i.p. administration of spermidine phosphate (320 mg kg) and spermine phosphate (160 mg kg) in mice

|               | Brain SPD (nmole mg) | Brain SPM (nmole mg) | SPM/SPD |
|---------------|----------------------|----------------------|---------|
| Control       | 91.52±4.33           | 104.04±4.41          | 1.14±0.07 |
| Spermidine    | 112.09±10.70         | 145.45±7.71*         | 1.30±0.08 |
| Spermine      | 137.67±3.19*         | 177.28±24.11*        | 1.29±0.20 |

Each value is the mean and standard error of 5-8 determinations, each of which involved pooled samples from two brains. *: p < 0.01 when compared with control (non-treated group).

Table 4: Brain spermidine and spermine contents 1 hr after i.p. administration of spermidine phosphate (320 mg kg) and spermine phosphate (160 mg kg) in mice

Table 5: Acute toxicity of the polyamines in mice

|               | LD50 (mg kg i.p.)    |
|---------------|----------------------|
| Spermidine    | 620 (500-769)        |
| Spermine      | 310 (200-480)        |

Mice treated with SPD and SPM were observed for 7 days. 30 animals per group.
and induced deep respiratory movement within one hour. Almost all mice injected with lethal doses of SPD and SPM died from 2 to 7 days (Table 5).

From the above results, SPM appears to have a considerably greater pharmacological activity than SPD. These results were summarized in Table 6.

### Table 6. Central pharmacological spectrum of the polyamines in mice

|                          | Spermidine | Spermine |
|-------------------------|------------|----------|
| 1. Depression of spontaneous motor activity | 180 (100-324) | 54 (28.4-102.6) |
| 2) Photo-cell counters method | 200 (118-340) | 88 (58.7-132.0) |
| 2. Anti-methamphetamine | 81 (42.4-153.0) |
| 3. Motor incoordination | 420 (318-544) | 210 (105-420) |
| 4. Potentiation of pentobarbital | 50 (26-95) | 10 (6.5-15.5) |
| 5. Conditioned avoidance test (avoidance) | 70 (48.6-100.8) |
| (escape)                  | 120        |
| 6. Writhing syndrome test | 115 (77-173) | 16 (9.9-25.4) |
| 7. Rectal temperature     | 230*       | 62*      |

* At this dose, rectal temperature fell 2°C.

### DISCUSSION

Onodera et al. (10) reported that SPD and SPM showed no effect in the isolated ileum, nor on the contractile response induced by acetylcholine or histamine, though the contraction induced by 5-HT was inhibited for 30% by SPM but not by SPD. It was also observed that SPD and SPM antagonized the ganglionic stimulant action of 5-HT or nicotine (10, 11). De Meis (11) reported that SPD and SPM relaxed frog skeletal muscle contracted either by acetylcholine, moderate increase in extracellular KCl concentration or electrical stimulation. Though Shaw (22) reported the effect of exogenous polyamines on the central nervous system (CNS), little is known of their physiological and pharmacological effects on the CNS.

The polyamines produced a decrease of SMA measured by the photo-cell counters method and the open-field test. Though this same author reported that after 100 mg/kg of SPD the animals revealed a hypothermia of 6°C or greater, 1-2°C of hypothermia was observed in a dose of 200 mg/kg of SPD and 25 mg/kg of SPM in our experiment. One obvious difference in doses of polyamines is that the latter author used hydrochloride salts of polyamines. In the hypothermia test as well as inhibitory effects on SMA, SPM had a more powerful action than SPD. It was observed that potentiation of hypnotic action of pentobarbital by SPD and SPM, and inhibition of writhing response by SPM occurred with small doses which did not produce depression of SMA. Shaw (22) found that the polyamines prolonged amylobarbitone sleeping time, and we also observed that the polyamines had the same effect. The potentiation of hypnotic action of pentobarbital by SPM was 5 times that of SPD. And inhibition of writhing response by SPM was much greater than
Motor incoordination of SPD and SPM was produced in more than double the dose which had an influence on SMA. LD50 for SPD and SPM was 620 mg/kg and 310 mg/kg, respectively.

In the present work, we found that brain SPM but not SPD was significantly increased by i.p. administration of SPD. On the other hand, i.p. administration of SPM produced marked increases in both SPD and SPM in the brain. As indicated in Table 4, the ratio of SPM/SPD in non-, SPD- and SPM-treated group was 1.14 ± 0.07, 1.30 ± 0.08 and 1.29 ± 0.20, respectively. Rosenthal and Tabor (2) reported that slight increases of SPD with marked elevations of SPM were observed in lung and kidney with an s.c. administration of SPM. Findings in the present experiment revealed that i.p. administration of SPM was increased not only SPM, but also SPD in the brain as well as in the peripheral tissues. Tadano (8) reported that the ratio of SPM/SPD changed in isolated aggressive mice but recovered to the normal level by either the administration of LiCl or transfer under the grouped circumstance.

The mechanism of action of the polyamines is as yet unclear. It should be clarified whether the effect of polyamines upon CNS is due to a direct or indirect action, e.g. the result of changes in brain catecholamines and serotonin with the administration of the polyamines.

REFERENCES

1) Shimizu, H., Kakimoto, Y. and Sano, I.: J. Pharmacol. exp. Ther. 143, 199 (1964)
2) Rosenthal, S.M. and Tabor, C.W.: J. Pharmacol. exp. Ther. 116, 131 (1956)
3) Tabor, H. and Tabor, C.W.: Pharmacol. Rev. 16, 245 (1964)
4) Shaw, G.G. and Pateman, A.J.: J. Neurochem. 20, 1225 (1973)
5) Shaskan, E.G., Harazsti, J.H. and Synder, S.H.: J. Neurochem. 20, 1443 (1973)
6) Shaskan, E.G. and Snyder, S.H.: J. Neurochem. 20, 1453 (1973)
7) Tadano, T., Onoki, M. and Kisara, K.: Folia pharmacol. jap. 70, 9 (1974) (in Japanese)
8) Tadano, T.: Folia pharmacol. jap. 70, 457 (1974) (in Japanese)
9) Sakurada, T.: Folia pharmacol. jap. 70, 629 (1974) (in Japanese)
10) Onodera, K., Unemoto, T., Miyaki, K. and Hayashi, M.: Arch. int. Pharmacodyn. Ther. 174, 491 (1968)
11) De Meis, L.: Am. J. Physiol. 212 (1), 92 (1967)
12) Ackermann, D. and Wasmuth, W.: Z. Physiol. Chem. 259, 28 (1938)
13) Tadasso, H., Fierz, H.F. and Vallenweider, H.H.: Helv. chim. Acta 27, 1384 (1944)
14) Parrot, T.: C. R. Soc. Biol. 142, 631 (1948)
15) Hall, C.S.: J. Comp. Psychol. 18, 385 (1934)
16) Dunham, N.W. and Miva, T.S.: J. Am. Pharm. Ass. 46, 208 (1958)
17) Kameyama, T. and Kisara, K.: Folia pharmacol. jap. 62, 317 (1966) (in Japanese)
18) Kameyama, T. and Kisara, K.: Advances in Neurrogical Sciences 10, 227 (1966) (in Japanese)
19) Endo, Y. and Ogura, Y.: Enrop. J. Pharmacol. 21, 293 (1973)
20) Satake, K., Okuyama, T., Ohashi, M. and Shindo, T.: J. Biochem. Tokyo 47, 654 (1960)
21) Litchfield, J.T. and Wilcoxon, F.: J. Pharmacol. exp. Ther. 96, 99 (1949)
22) Shaw, G.G.: Arch. int. Pharmacodyn. Ther. 198, 36 (1972)