CHANGES IN INGESTIVE BEHAVIOR, SERUM GLUCOSE AND FREE FATTY ACIDS CONCENTRATIONS IN RATS FOLLOWING INTRACEREBROVENTRICULAR INJECTION OF SPERMINE

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Abstract—We examined the changes in ingestive behavior, serum glucose (Glc) and free fatty acids (FFA) concentrations in male rats following intracerebroventricular (i.c.v.) injection of spermine (SPM). In satiated rats, over a 53.3 nmol of SPM suppressed feeding and drinking behavior in a dose-dependent manner. The median suppressive dose was 90.8 nmol for feeding behavior and 68.3 nmol for drinking behavior. Spermidine also suppressed ingestive behavior but the potency was appreciably weak compared to that of SPM, and the occurrence of the maximal suppression was observed 2–3 days later than that of SPM. The most significant anorexia and adipsia induced by SPM appeared between 16 and 36 hr after i.c.v. administration. SPM (180 nmol) produced a biphasic increase in serum Glc concentration. The 1st peak was at 1 hr and the other peak was 24 hr after the dosing. The same dose of SPM elevated serum FFA concentrations gradually, and the maximal increase appeared 24 hr after the injection. As less than a 120 nmol dose of SPM did not alter serum Glc and FFA concentrations, there seems to be no causal relation between SPM-induced anorexia, and changes in serum Glc and FFA concentrations. Nevertheless, the findings that a very small dose of SPM produced anorexia and adipsia support the possibility that SPM may play some functional role in the brain.

Polyamines, spermine (SPM), spermidine (SPD) and putrescine (PUT) are widely distributed in the mammalian brain (1–5) as well as in almost every living material (6). In the last 30 years, a large number of experimental evidence has shown that polyamines, particularly SPM, may play some physiological roles in the brain (7). Polyamines produce centrally directing effects such as hypomotility and hypothermia (8–11). Anderson et al. reported that SPD injected into the lateral cerebroventricles of mice produced anorexia, adipsia and body-weight loss (10), and that a very small dose of polyamines which does not produce other actions does suppress feeding and drinking behavior of rats.

We found that intracerebroventricular (i.c.v.) injection of SPM produced a significant body-weight loss in mice (11), and
that a 40 μg dose of SPM (as the tetrahydrochloride salt), which was effective in producing insomnia, also suppressed feeding behavior in rats (12). In addition, SPM has been shown to produce hyperglycemia (13, 14).

The present work was undertaken to investigate the anorectic effect of SPM in detail, and to clarify the correlation between the anorexia and the changes in serum glucose (Glc) and free fatty acids (FFA) concentrations which are considered to be most important in the regulation of feeding behavior (15). Some of the data obtained were included in a preliminary report (16).

MATERIALS AND METHODS

Animals and cannula implantation: All the experiments were performed on male Wistar rats weighing 250–300 g at the time of cannula implantation. Each rat was anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and a stainless-steel guide cannula with an inner obturator (Fig. 1) was stereotaxically implanted into the left lateral cerebroventricle, according to the atlas of Pellegrino et al. (17). Over one week was allowed to elapse before the testing.

Procedures for a measurement of ingestive behavior: Each rat was housed in a wire-mesh cage (26×36×19 cm) in a room that was illuminated from 9:00 to 21:00 and was kept at 22±2°C. Powdered food (CE-2, Clea Japan, Inc.) and tap water were freely provided by using a glass cup and a graduated glass tube, respectively. Food and water intake, and body weight were measured daily at 11:00. Each rat was conditioned to the type of procedure to which they would be exposed on the time of i.c.v. injection. When food and water intake became constant, each rat was removed from the home cage to a large open cage (34×46×18 cm), the inner obturator was removed, and a 10 μl volume of each drug solution was infused via an injection cannula connected to Hamilton microliter syringe through a polyethylene tube. The injection cannula was kept in the guide cannula for 30 sec so that the drug solution would diffuse sufficiently.

In case of experiments on the food- and water-deprived rats, each animal was trained to a 4 hr (11:00–15:00) per day feeding and drinking schedule until the body weight was either stabilized or increased. Each rat was conditioned sufficiently to the type of handling to which it would be exposed at the time of a drug administration, and then SPM was given i.c.v. Cumulative food and water intake during 4 hours, and body weight were measured at 15:00. To determine the peak time of SPM-induced anorexia and adipsia, the rats were grouped into 6 and SPM (180 nmol) was given i.c.v. to each group at 11:00, 15:00, 19:00, 23:00, 3:00 and 7:00, respectively.

Procedures for the determination of serum Glc, FFA and immunoreactive insulin concentrations: Each rat was housed and handled

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**Fig. 1.** Instruments for the intracerebroventricular injection. A. Guide cannula. B. Inner obturator. C. Injection cannula. a) Stainless steel hypodermic needle (23 ga.) cut to 5 mm length. b) Stainless steel wire (0.8 mm dia.) to grip. c) Cap to close a guide cannula up tightly molded out of dental cement by pouring into a guide cannula. d) Stainless steel wire (0.3 mm dia.) to close a guide cannula. e) Stainless steel pipe (0.6 mm dia.) to connect a polyethylene tube (1.0 mm dia.) tightly. f) Stainless steel pipe (0.3 mm dia.) to infuse a drug solution.
as in the case of the behavioral study. Body weight and food intake were monitored daily. On the day of determination, food and water were removed 3 hours prior to the blood sampling. Considering the circadian variation of Glc (18) and FFA (19), at just 14:00, blood samples from each rat which has been given the i.c.v. injection of SPM (180 nmol) were collected following decapitation and all the samples were centrifuged. Determination of each substance in the serum was as follows: o-toluidine boric acid method (20) for Glc, NEFA test Wako (Wako Pure Chemical Industries, LTD.), a modification of the method of Laurell and Tibbling (21) for FFA and radioimmunoassay using Insulin Eiken kit (Eiken, ICL.) based on a double antibody method for immunoreactive insulin (IRI). In case of the experiments in which the effects of various doses of SPM 24 hr after the injection on serum Glc and FFA concentrations were estimated, the rat was deprived of food and water for 24 hours in order to eliminate the influence of SPM-induced anorexia and adipsia.

Procedures for the confirmation of a cannula placement: At the end of the experiment, the cannula placement was confirmed by infusing 0.2% solution of Evans blue dye in a volume of 10 μl and observing the distribution in the cerebroventricular system.

Drugs: Drugs used were spermine tetrahydrochloride and spermidine trihydrochloride (Nakarai Chemicals, LTD.). Each drug was dissolved in sterile Ringer’s solution, the pH value was adjusted to 7.4±0.2 by an addition of 0.5 N-NaOH solution and it was then given i.c.v. in a volume of 10 μl. The same volume of sterile Ringer’s solution adjusted to the same pH value as a drug solution was injected i.c.v., as a vehicle control.

Statistics: The differences between the Ringer’s solution-treated group and each drug-treated group were evaluated by the Student’s t-test (two-tailed).

RESULTS

Effects of SPM and SPD on feeding and drinking behavior in satiated rats: SPM suppressed feeding behavior dose-dependently (Fig. 2, top). The smallest dose of SPM (53.3 nmol) had no effect on a 24 hr-
intake following i.c.v. injection, but suppressed the next 24 hr-intake. Over an 80 nmol dose of SPM suppressed feeding behavior during 7 days after the dosing. The percent suppression of feeding behavior during 24 hours immediately after each dose of SPM was as follows: 10% for 53.3 nmol, 49.7% for 80 nmol, 68.8% for 120 nmol and 86.7% for 180 nmol. The median suppressive dose calculated from these values was 90.8 nmol (60.4–136.4 nmol as the 95% confidence limit). Drinking behavior was also suppressed in parallel with feeding behavior (Fig. 2, middle). The percent suppression of 24 hr-intake immediately after the injection of each dose of SPM was as follows: 19.6% for 53.3 nmol, 62.2% for 80 nmol, 86.5% for 120 nmol and 97.0% for 180 nmol. Calculating from these values, the median suppressive dose was 68.3 nmol (21.0–221.3 nmol as the 95% confidence limit). Drinking behavior was also suppressed in parallel with feeding behavior (Fig. 2, middle). The percent suppression of 24 hr-intake immediately after the injection of each dose of SPM was as follows: 19.6% for 53.3 nmol, 62.2% for 80 nmol, 86.5% for 120 nmol and 97.0% for 180 nmol. Calculating from these values, the median suppressive dose was 68.3 nmol (21.0–221.3 nmol as the 95% confidence limit). Body weight was reduced by all doses of SPM (Fig. 2, bottom). When over an 80 nmol dose of SPM was given, rats appeared to be sedated, however, they respond readily to external stimuli such as a chattering sound.

Figure 3 shows the effects of SPD on feeding behavior (top), drinking behavior (middle) and body weight (bottom). Over an 324 nmol dose of SPD suppressed feeding and drinking behavior. The most potent suppression of such behavior was observed on the 4th day, and then the percent suppression of feeding and drinking behavior was: 36.3% and 33.5% for 324 nmol, 83.8% and 87.3% for 388.8 nmol, respectively. Body weight was also reduced in parallel with ingestive behavior. There were no behavioral changes after a 270 nmol dose of SPD, but sedation was to some extent apparent when a 324 nmol dose of SPD was given. The largest dose of SPD (388.8 nmol) produced a significant sedation and ataxia in all the rats (8 rats) and hematuria in 5 out of 8 rats. Four rats of these 5 rats died by the 7th day after the dosing.

Effects of SPM on feeding and drinking behavior of rats under limited feeding and drinking schedule: Figure 4 shows the effects of SPM on feeding behavior (top), drinking behavior (middle) and body weight (bottom) of rats adapted to 4 hr per day-feeding and -drinking schedule. During 4
hours immediately after the i.c.v. injection (day 0 in Fig. 4) the suppressive effects induced by all doses of SPM (53.3–180 nmol) on feeding and drinking behavior were very weak, however, the most potent suppression of both behaviors appeared on day 1. The effect of 180 nmol of SPM was almost equal to that of 120 nmol of SPM. Since there was a very weak effect during 4 hours immediately after the dosing, the time course of the occurrence of anorexia and adipsia following the injection of 180 nmol of SPM was examined (Fig. 5). The marked feeding and drinking deficits appeared between 4 and 8 hr after the injection. The food and water intake during this period was 10.8% and 7.8%, respectively. The suppressive effect was slightly weakened during the next 4 hours, but a subsequent reversion was seen. The most potent suppression was observed between 16 and 36 hr after the dosing.

Effects of SPM on serum Glc, FFA and IRI concentrations: SPM (180 nmol) produced a biphasic increase in serum Glc concentration 1 and 24 hr after the injection (Fig. 6). Only the latter increase was statistically significant compared to the Ringer's solution treated group in the 3 hr-food and -water deprived rats, and was also dose-dependent in the 24 hr-food and -water deprived rats (Table 1). However, an 80 nmol dose of SPM which was sufficiently effective on ingestive behavior did not alter serum Glc concentrations.

SPM (180 nmol) did not alter serum FFA concentrations up to 3 hr, but subsequently serum FFA concentration was gradually increased and the maximal increase was obtained 24 hr after the injection (Fig. 7). The increase in serum FFA concentration which appeared at 24 hr was statistically significant in the 3 hr-food and -water deprived rats, and a dose-response relationship was also observed in the 24 hr-food and -water deprived rats (Table 2). However, only the largest dose of SPM (180 nmol) produced a marked increase in serum FFA concentrations.

SPM (180 nmol) increased serum IRI concentration to 56.2±6.1 μU/ml 24 hr after the dosing, but there was no statistical
significance compared to findings in the case of Ringer's solution (37.3±5.4 μU/ml) (Fig. 8).

DISCUSSION

It was confirmed that SPM suppressed feeding and drinking behavior when a very small dose such as 53.3 nmol (10.8 μg as the free base) was given. The dose of 53.3 nmol does not seem to be an unphysiological large amount in the brain of adult rats which weighs 1.7–1.9 g, since the concentration of
Table 1. Serum glucose concentrations after the intracerebroventricular injection of spermine in 3 hr- and 24 hr-food and water deprived rats

| Condition of rat               | Time after injection | Treatment         | Number of rats | Serum glucose concentrations (mg/dl) Mean±SEM | % increase |
|-------------------------------|---------------------|-------------------|----------------|---------------------------------------------|------------|
| 3 hr-food and water deprivation | 1 hr                | Ringer’s solution | 5              | 145.8±3.7                                   | —          |
|                               |                     | Spermine 180 nmol | 8              | 155.5±7.0                                    | 6.7        |
|                               | 24 hr               | Ringer’s solution | 6              | 143.8±4.6                                    | —          |
|                               |                     | Spermine 180 nmol | 9              | 181.1±6.3**                                  | 25.9       |
| 24 hr-food and water deprivation | 24 hr              | Ringer’s solution | 6              | 113.6±3.5                                    | —          |
|                               |                     | Spermine 80 nmol  | 4              | 119.0±1.6                                    | 4.8        |
|                               |                     | Spermine 120 nmol | 4              | 130.3±4.0*                                   | 15.1       |
|                               |                     | Spermine 180 nmol | 4              | 144.2±6.5**                                  | 26.9       |

*p<0.02 compared to Ringer’s solution. **p<0.01 compared to Ringer’s solution.

Fig. 7. Changes in serum free fatty acids (FFA) concentration after spermine (180 nmol, i.c.v.). Each value represents mean ± SEM. The number of rats for each point is given in each parenthesis.

Table 2. Serum free fatty acids (FFA) concentrations after the intracerebroventricular injection of spermine in 3 hr- and 24 hr-food and water deprived rats

| Condition of rat               | Time after injection | Treatment         | Number of rats | Serum FFA concentrations (μEq/l) Mean±SEM | % increase |
|-------------------------------|---------------------|-------------------|----------------|------------------------------------------|------------|
| 3 hr-food and water deprivation | 24 hr               | Ringer’s solution | 6              | 323.9±75.7                                | —          |
|                               |                     | Spermine 180 nmol | 9              | 563.6±45.7*                               | 71.3       |
|                               | 24 hr               | Ringer’s solution | 6              | 461.0±47.0                                 | —          |
|                               |                     | Spermine 80 nmol  | 4              | 486.3±52.2                                 | 5.5        |
|                               |                     | Spermine 120 nmol | 4              | 506.0±14.2                                 | 9.8        |
|                               |                     | Spermine 180 nmol | 4              | 732.6±51.6**                               | 58.9       |

*p<0.02 compared to Ringer’s solution. **p<0.01 compared to Ringer’s solution.
SPM in the rat whole brain has been shown to be 145.2 nmol/g (22) or 212.5 nmol/g (1).

Fig. 8. Effect of spermine (180 nmol, i.c.v.) on serum immunoreactive insulin (IRI) concentration 24 hr after the dosing. Each value represents mean ± SEM. The number of rats for each group is given in each parenthesis.

SPM in the rat whole brain has been shown to be 145.2 nmol/g (22) or 212.5 nmol/g (1).

SPD also suppressed feeding and drinking behavior, but the potency was so weak that over a 324 nmol dose was required for a significant suppression. Moreover, the maximal effect was observed 2–3 days later than that of SPM. Anderson et al. have also reported that a 50 μg dose of SPD (344.3 nmol) produced marked anorexia and adipsia for more than 5 days in mice (10). Thus, SPD seems to exert a nearly equal effect on rats and mice. The largest dose of SPD (388.8 nmol) produced less potent anorexia and adipsia than did SPM, nevertheless half of rats died with an accompanying ataxia and hematuria.

Regarding these different mode of actions between SPM and SPD, it seems likely that each substance plays a different role in the brain. Ingoglia et al. investigated the axonal transport of polyamines, SPM, SPD and PUT in goldfish optic nerves, and found that the transport rate in normal nerves was in the order of SPM>SPD>PUT. In regenerating nerves the rate was PUT>SPD>SPM. Based on these results, they suggested that each polyamine has a different role in the brain and that SPM is primarily involved in the regulation of neuronal activity (23, 24). In fact, PUT has been shown to produce a very weak anorexia compared to SPM (25).

It is of interest to note that the appearance of the maximal inhibitory effect of SPD was later than that of SPM. This result suggests that SPM synthesized from exogenously injected SPD (26) is also involved in the SPD-induced anorexia and adipsia.

In addition to the behavioral deficits, serum Glc and FFA concentrations were elevated with SPM administration. The biphasic increase in serum Glc concentration is most interesting, since SPM-induced anorexia was also dualistic in experiments under condition of a limited feeding schedule. However, as an 80 nmol dose of SPM, which suppressed feeding behavior, had almost no effect on serum Glc concentration, it is reasonable to assume that the hyperglycemia is not the cause of the anorexia, but rather that it is produced independently by SPM in parallel with the anorexia. Anderson and Shaw reported that SPM produced a significant hyperglycemia 1 hr after the i.c.v. injection in rats (14). We also found that SPM slightly increased serum Glc concentrations, at the same hour. The difference in the extent of hyperglycemia between the two observations is probably due to the fact that they used a 4 fold dose of SPM. They also found that SPM did not produce hyperglycemia in the adrenal-demedullated rats. Therefore, the increase in serum Glc concentrations 1 hr after SPM may result from stress to which the rats were exposed at the time of injection. In the present experiment, however, considering the findings that each rat was well adapted to the type of procedure to which they would be exposed at the time of the i.c.v. injection, and that the i.c.v.
injection of Ringer's solution did not substantially change serum Glc concentration, the hyperglycemia seen at 1 hr after SPM may not be due to stress but rather to the effect on the central nervous system.

The marked increase in serum Glc concentrations 24 hr after SPM is not due to the inhibition of insulin secretion, since serum IRI concentration increased at the same time.

The increase in serum FFA concentrations observed at 16 or 24 hr after SPM does not appear to be related to the anorexia, but rather may be due to the action of SPM on the central nervous system, since SPM elevated serum FFA concentrations even in the 24 hr-food and -water deprived rats.

Regarding the findings of Halliday and Shaw (26), a large amount of SPM injected i.c.v. was distributed in the caudate nucleus, hypothalamus and medulla oblongata. The hypothalamus is involved in the regulation of feeding (27) and drinking (28) behavior, blood Glc and FFA levels (29). It is generally acknowledged that the ventromedial hypothalamic nucleus (VMH) and the lateral hypothalamic area (LH) play reciprocal roles in the regulation of feeding behavior (27) and Glc metabolism (29, 30). Electrical stimulation of the VMH causes suppression of eating (31, 32), hyperglycemia (29, 30, 33) and lipolysis (34). Reverse effects such as an increase in food intake (31, 32) and hypoglycemia (29, 30) have been observed when the LH was stimulated. Drinking behavior has also been elicited by electrical stimulation of the LH and inhibited by a lesion of that area (28). From these findings, the VMH-visceral nervous system seems to be more dominant than the LH-vagus system 24 hr after the i.c.v. injection of SPM. However, firm conclusions on the site of action cannot be drawn, since SPM did not inhibit an increase in serum IRI concentration such as is seen during electrical stimulation of the VMH (33), and some extrahypothalamic regions may also be important in the regulation of ingestive behavior (35) and blood Glc levels (36).

The biphasic effects of SPM on ingestive behavior and serum Glc concentration are interesting, since SPM binds cell membranes and the nucleus (37). The 1st phase of these effects of SPM may be a membrane-mediated action, as SPM exerts effects on membranes or membrane-bound enzymes, cholinesterase (38) and Na,K-ATPase (39) in the brain. These findings suggest that SPM may play some functional roles in neurotransmission. The nearly 50% distribution in the synaptosomal fraction (40), the existence of a high affinity uptake system (41, 42), a depolarization-induced and Ca²⁺-dependent release from the rat cerebral cortex slices (43) and influence on a neuronal firing (44) support the suggestion.

The 2nd phase of SPM-induced anorexia, adipsia and hyperglycemia might be produced by an effect on the nucleic acid in the nucleus and/or cytoplasm, since SPM has been acknowledged to act on nucleic acids (37, 45) and to be distributed in the nuclear fraction of rat brain cortex homogenate (40). Moreover, SPM is a natural component of the yeast phenylalanine tRNA molecule (46). The necrotic lesions in the mouse brain after the i.c.v. injection of SPM (10) may be produced through critical cellular metabolic changes. However, it has not been determined whether the minimal effective dose of SPM, 53.3 nmol, produce such lesions in the rat brain.

Metabolites of SPM such as spermic acid (47) are probably not involved in the 2nd phase, because the half-life for SPM in the brain is extremely long, 92 days (48) or 42 days (49).

Our findings support the possibility that SPM may have physiological roles in the brain.
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