Alteration of ingestive behaviours by nucleus accumbens in normal and streptozotocin-induced diabetic rats

G K Pal, Pravati Pal & Madanmohan

Department of Physiology, Jawaharlal Institute of Post-graduate Medical Education and Research, Pondicherry 605006, India

e-mail: gopalpravati@sify.com

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Twenty-four hour basal food and water intakes were recorded in Wistar rats. Diabetes was produced in a group of rats by injecting streptozotocin (STZ, 75 mg/kg, b.w., IP) and their post-diabetic basal food and water intakes were recorded. Noradrenaline (2 μg) and dopamine (2 μg) were injected separately into the nucleus accumbens through the implanted cannula in non-diabetic and diabetic animals and their 24 hr food and water intakes were recorded. Food and water intakes were also recorded following bilateral electrolytic lesions of nucleus accumbens in both the groups of rats. In diabetic rats, basal food and water intakes were significantly increased in comparison to basal intakes of non-diabetic rats. Following injection of noradrenaline, a significant increase in water intake but not food intake was seen in non-diabetic rats, whereas food and water intakes remained unchanged in diabetic rats. Following injection of dopamine, a significant increase in food and water intakes was observed in non-diabetic rats, whereas dopamine-induced increase in food intake was absent in diabetic rats. The bilateral lesions of nucleus accumbens resulted in a significant inhibition of food and water intakes in non-diabetic rats, whereas inhibition of water intake without change in food intake observed in diabetic rats. However, no difference was observed in the pattern of change in water intake following lesions or dopamine injections between non-diabetic and diabetic rats, whereas difference was observed for food intake. The results suggest that nucleus accumbens activity changes for food intake, but not for water intake in diabetes.

Nucleus accumbens has been reported to be involved in regulation of food and water intakes in rats. Injection of dopamine into nucleus accumbens facilitates water intake in a dose dependent manner reaching its maximum at 2μg dose of the chemical. Dopamine-facilitated water intake is associated with increase in food intake and this dopamine-facilitated food and water intake is mediated by D2 receptors. Intracerebral injection of noradrenaline into nucleus accumbens in rats increased water intake without affecting food intake in a dose dependent manner reaching its maximum at the 2μg dose of the chemical. Noradrenaline-facilitated water intake is mediated by alpha receptors. Injection of angiotensin-II into nucleus accumbens in rats increases water intake and is partly dependent on the intact dopaminergic receptors.

Diabetes mellitus is a common endocrine disorder associated with increased food and water intakes. Increased food intake in diabetes is due to facilitation of the activities of lateral hypothalamus (the feeding center), which occurs due to the removal of inhibitory influence of ventromedian hypothalamus (the satiety center) on it. Though there is no report of insulin-sensitivity of nucleus accumbens, as it is closely linked with hypothalamus, one could expect changes of the influence of this nucleus on food and water intakes in diabetes. Therefore, using diabetes model, the present study has been carried out to assess the effects of lesion of nucleus accumbens, and injection of dopamine and noradrenaline into it on food and water intakes.

Materials and Methods

Institute bred 180 male albino rats of Wistar strain weighing 250-300 g body weight were used. Each animal was kept in a separate cage. The temperature of the room in which animals were caged was between 25° and 28°C. Animals were exposed to 24 hr natural light-dark cycle. Food and water were provided ad libitum. Basal food and water intakes were measured as described in earlier experiment.

Induction of diabetes—Streptozotocin (STZ)-induced diabetes was produced in a group of 10 animals. STZ (Sigma Chemicals, St. Louis, MO) was prepared as 1% solution in citrate buffer (pH 4.5) and was administered i.p. in a dose of 75 mg/kg, b.w. (a single injection of STZ that causes preferential lysis of β cells of pancreas is adequate to produce diabetes in animal models in 72 hr). For blood glucose estimation...
(to confirm diabetes), 0.5ml blood was collected from the tail veins of the rats. Non-fasting blood glucose was measured by glucose oxidase method a day before the injection of STZ and daily for 5 consecutive days from the fourth day after the injection of STZ during and after which different experiments were performed. Basal food and water intakes were also measured in diabetic animals.

Preparation of dosage of chemicals—Stock solutions of noradrenaline (N.I. Pharma, Calcutta), dopamine (N.I. Pharma, Calcutta), sulpiride (TOCRIS, UK), and SCH 23390 hydrochloride (TOCRIS, UK) were prepared separately by proportionately dissolving the chemicals in different solvents in such a way that 1 μl of each solution contained 2 μg of chemicals. Noradrenaline and dopamine were dissolved in normal saline, sulpiride was dissolved in ethanol and SCH 23390 hydrochloride (SCH) was dissolved in distilled water. The stock solutions were preserved in the refrigerator. However, the temperature of chemicals was brought to the room temperature prior to injection into the nucleus accumbens.

Experimental protocol—Following 3 experimental protocols were used in different groups of animals. Cannulations were performed as described earlier 3.

Group 1: Noradrenaline (2 μg dissolved in 1 μl normal saline) was injected into the nucleus accumbens in non-diabetic (n=10) and diabetic (n=10) groups through the implanted cannula at 1400 hrs following which 24 hr food and water intakes were recorded. The chemical was injected slowly from a microliter syringe (Top S. M. Co., Bombay) fitted into a Continuous Slow Injector (INCO), over a period of 2 min. As the strength of the solution containing 2 μg chemical in 1 μl normal saline is 1.1%, food and water intakes were also recorded following injection of 1 μl of 1.1% saline solution separately in non-diabetic animals (n=10, this served as control group).

Group 2: Dopamine (2 μg dissolved in 1 μl normal saline) was injected into the nucleus accumbens in non-diabetic (n=10) and diabetic (n=10) groups through the implanted cannula at 1400 hrs following which 24 hr food and water intakes were recorded. Food and water intakes were also measured following injection of 2 μg dose sulpiride, a central D2 dopamine receptor antagonist, and SCH, a central D1 dopamine receptor antagonist, in the same animals on separate days. Each dose of the chemical was injected slowly from the microliter syringe. Food and water intakes were also recorded following injection of 1 μl ethanol and distilled water separately in non-diabetic animals (n=10) that served as control groups for sulpiride and SCH respectively.

Group 3: Stainless steel electrodes were prepared and insulated (except the tip of the electrodes) by perspex material dissolved in chloroform. The electrodes were inserted bilaterally into the nucleus accumbens in 20 animals (10 non-diabetic and 10 diabetic) by stereotaxy using the coordinates of Konig and Klippel 12. The electrolytic lesions of the nuclei were produced by passing an anodal current of 1 mA for 15 sec with the help of a lesion maker (INCO). After a recovery period of 2 days the food and water intakes were recorded for 7 consecutive days in all animals to record the mean post-lesion food and water intakes. The food and water intakes of the post-lesion period were compared with their pre-lesion values. Cannulations and site of lesions were confirmed as described earlier 3. The values are presented as mean ± SE. Significance of differences was tested by Student’s ‘t’ test.

Results and Discussion
The basal food and water intakes of all the animals were found to be 14.30±0.26 g/day and 21.37±0.23 ml/day, respectively. Diabetes was produced in 30 animals following single intraperitoneal injection of STZ, which was confirmed by sustained rise in blood glucose level. The pre-diabetic blood glucose was 64.9±1.72 mg/dl and the post-diabetic blood glucose was 331.4±3.47 mg/dl. Water and food intakes were also significantly increased in diabetic animals (compared with their pre-diabetic values). The basal mean post-diabetic food and water intakes were 18.42±0.27 g/day and 26.52±0.28 ml/day, respectively.

In group 1, a significant increase in water intake (27.35±0.64 ml) without change in food intake was observed following intracerebral injection of 2 μg dose of noradrenaline into the nucleus accumbens in non-diabetic animals (Table 1). But, no change in food and water intake was observed following the injection of noradrenaline into the nucleus in diabetic animals. No significant change in food and water intakes was also observed following the injection of 1.1% saline in control animals.

In group 2, a significant increase in food (21.14±0.67 g) and water intake (28.48±0.24 ml) was observed following intracerebral injection of 2 μg dose dopamine into the nucleus accumbens in non-
diabetic animals (Table 2). But, increase in water intake (though the level of significance is less than that of the non-diabetic rats) without change in food intake was observed following the injection of dopamine into the nucleus in diabetic animals. Following injection of 2μg dose of sulpiride, a D₂ dopamine receptor antagonist, food and water intakes were significantly inhibited in non-diabetic animals, whereas water intake was suppressed without change in food intake in diabetic animals (Table 2). There was no significant change in food and water intakes following the injection of 2μg dose of SCH, a D₁ dopamine receptor antagonist. Food and water intake also remained unchanged in control animals.

In group 3, a sustained and significant decrease in food and water intake was observed following bilateral electrolytic lesions of nucleus accumbens in non-diabetic animals. Significant decrease in water intake without significant change in food intake was observed in diabetic animals (Table 3).

In the present study, water intake increased significantly without affecting food intake when noradrenaline was injected into nucleus accumbens in non-diabetic rats, which is consistent with the findings of previous study. But, noradrenaline does not facilitate water intake when injected into the same nucleus in diabetic animals. This may be due to the increase in basal water intake to its maximum in diabetic rats, as there is no significant difference between post-diabetic basal water intake and water intake following injection of noradrenaline in non-diabetic rats. Therefore, noradrenaline could not facilitate water intake further in these animals. Dopamine injected into nucleus accumbens facilitated food and water intake in non-diabetic rats. This is also consistent with our earlier observations. But, injection of dopamine into nucleus accumbens in diabetic rats has not facilitated food intake, whereas water intake increased moderately. The moderate increase in water intake in diabetic rats following injection of dopamine has occurred due to the reason that the post-diabetic basal water intake had not reached its maximum in these animals. This is evidenced from the observation that increase in water intake following injection of dopamine in non-diabetic rats is significantly higher than the basal water intake in diabetic rats. Therefore, injection of dopamine resulted in further increase in water intake to its ceiling point in diabetic rats. The dopamine facilitated water intake is mediated by D₂ dopamine receptors, as sulpiride, a D₂ receptor antagonist inhibited water intake in both non-diabetic and diabetic rats. It is also clear that the dopamine facilitated food and water intake, is not mediated by D₁ dopamine receptors, as SCH, a D₁ dopamine receptor antagonist does not alter food and water intake in both non-diabetic and diabetic rats. It also appears

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<th>Table 1 — Change in 24 hr food intake (g) and water intake (ml) following injection of 2 μg noradrenaline (NA), into nucleus accumbens in non-diabetic and diabetic rats</th>
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<td><strong>Values are mean ± SE from 10 animals in each group</strong></td>
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<td>*P &lt; 0.001; # Basal means the basal 24 hr food intake and water intake of animals before injection of NA.</td>
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<th>Table 2 — Change in 24 hr food intake (g) and water intake (ml) following injection of 2 μg dopamine (DA), 2 μg of sulpiride (SP), and 2 μg of SCH 23390 hydrochloride (SCH), into nucleus accumbens, in non-diabetic and diabetic rats</th>
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| *P values: * < 0.01; ** < 0.001; # Basal means the basal 24 hr food and water intake of animals before injection of DA, SP and SCH.
from these observations that the nucleus accumbens activity for water intake has not changed in diabetes, as the pattern of alteration of water intake in non-diabetic and diabetic rats remained same following lesion of the nucleus following injections of dopamine and dopamine antagonists into the nucleus. Similarly, one could also argue that dopamine has not increased food intake in diabetic rats because the food intake (post-diabetic basal intake) has reached its maximum in diabetic animals. But, this postulation is not applicable for food intake, because the dopamine-induced food intake in non-diabetic rats is significantly higher than the post-diabetic basal food intake. This indicates that the nucleus accumbens activity for food intake has altered in diabetes. However, further studies are required to establish the mechanism that results in such alteration of food intake in diabetes.

Sustained and significant decrease in 24 hr food and water intakes following bilateral lesions of nucleus accumbens in non-diabetic rats indicates that this nucleus has a stimulatory effect on feeding and drinking behaviour in rats. This result is consistent with our earlier studies. However, absence of inhibition of food intake following lesions of nucleus accumbens in diabetic rats indicates that this nucleus does not facilitate feeding in diabetes. But, as water intake is significantly and proportionately inhibited in the same group of rats, it indicates that influence of nucleus accumbens on water intake does not change in diabetes.

Unlike feeding center, which is primarily localized in lateral hypothalamus, the thirst centers are widely scattered in different areas of the brain. Lesion of nucleus accumbens decreases water intake and injection of catecholamines and angiotensin into this nucleus increases water intake. Therefore, we propose nucleus accumbens as one of the thirst centers in the brain. Lesion of nucleus accumbens significantly inhibits water intake in both normal and diabetic rats. In diabetic rats decreased water intake was not associated with similar change in food intake, which indicates the strong dipogenic nature of the nucleus accumbens. This is further supported by the evidence that the injection of norepinephrine into this nucleus in normal rats increases water intake without affecting food intake. Therefore, noradrenaline-facilitated water intake is a primary polydipsia (increase in water intake is not associated with increase in food intake). Further investigations are required to establish the role of nucleus accumbens in the regulation of drinking behaviour in diabetic animal models.

Acknowledgement

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