Pharmacology Biochemistry and Behavior, Vol. 44, pp. 601-604, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

Effects of Streptozotocin-Induced Diabetes on Dopaminergic Functioning in the Rat: Analysis of Yawning Behavior

JEREMY P. W. HEATON[1] AND SHAWN J. VARRIN

Human Sexuality Group, Department of Urology, Faculty of Medicine, Queen's University, Kingston, Ontario K7l 3N6, Canada

Received 1 June 1992

HEATON, J. P. W. AND S. J. VARRIN. *Effects of streptozotocin-induced diabetes on dopaminergic functioning in the rat: Analysis of yawning behavior.* PHARMACOL BIOCHEM BEHAV 44(3) 601-604, 1993.—Apomorphine, a dopamine receptor agonist, causes yawning in rats. It has been suggested that the analysis of yawning behavior provides an index of dopamine autoreceptor function. Dopamine turnover in the substantia nigra of diabetic rats has been shown to be decreased following administration of amphetamine or apomorphine (17,21). Yawning behavior after 4 weeks of streptozotocin (STZ)-induced diabetes in Wistar rats was significantly lowered when compared with their age-matched normal controls. Yawning behavior was not further diminished after an 8-week duration of diabetes mellitus; however, a significant recovery in yawning was seen by 20 weeks of diabetes. Yawning in rats after 20 weeks of STZ-induced diabetes mellitus is not significantly different from that seen in normal control rats. The results suggest that in STZ-induced diabetes of only 4 weeks duration a measurable change in the substrate for yawning has occurred.

Dopamine Yawning Apomorphine Autoreceptor

APOMORPHINE (APO), a dopamine (DA) receptor agonist, has been shown by us (7-9) and others (5,22) to reliably produce bouts of yawning in rats when administered in microgram quantities. The ability of APO to induce yawning behavior in rats is said to be a result of its interaction with dopaminergic autoreceptors (5,22,20) although some authors have questioned the hypothesis of autoreceptor mediation of yawning (19). Electrophysiological techniques show that the stimulation of dopaminergic autoreceptors reduces the tonic firing of neurons (18). Activation of autoreceptors located on dopaminergic terminals causes decreased synthesis and release of DA from these terminals (4). Low doses of DA or the DA agonist APO given parenterally act selectively on midbrain DA cell autoreceptors without stimulating postsynaptic DA receptors (15,18). Thus, it is felt that the ability of APO to induce yawning behavior may provide an index of central DA autoreceptor function (5).

Numerous studies documented alterations in yawning behavior as a result of various pharmacologically induced neurochemical challenges; for instance, yawning behavior has been shown to be affected by such substances as metoclopramide (6), haloperidol (5), sulpiride (5), calcium channel blockers (2), oxytocin antagonists (3), and several cholinergic antagonists (22). Alterations in yawning behavior as a function of disease processes have not been investigated.

Diabetes mellitus alters many of the central neurotransmitter mechanisms; however, its effects on the central DA system are not well established. DA turnover in the synaptosomes prepared from striata and in the limbic forebrain has been shown to be decreased in diabetic rats following administration of amphetamine or APO (17,21). Peripheral alterations have also been shown to occur; both norepinephrine and DA were documented to be decreased in the cardiovascular system of long-term diabetics at postmortem (14). Thus, evidence is sparse outlining alterations in dopaminergic functioning in diabetes mellitus; moreover, to the best of our knowledge the effects of diabetes upon the central substrate necessary to stimulate APO-induced yawning behavior have yet to be examined.

The present study examines the effects of short-term (4 weeks) and long-term (up to 20 weeks) streptozotocin (STZ)-induced diabetes mellitus in producing significant alterations in dopaminergically mediated yawning behavior. Alterations in the expression of yawning behavior are postulated to represent alterations in the substrate (neurochemical or other) responsible for yawning.

[1] To whom requests for reprints should be addressed.

METHOD

Male Wistar rats (230–260 g) were obtained from Charles River laboratories Canada Inc. (St. Constant, Quebec). Animals were housed in individual stainless steel wire cages with a 12 L : 12 D cycle, the dark (waking) cycle commencing at 1850 h. The ambient room temperature was maintained at 24°C and the relative humidity at 50%. After 1 week of handling and acclimatization, rats were randomly divided into two groups. One group was rendered diabetic with an IP injection of STZ (65 mg/kg) dissolved in a saline citrate (0.1 M citrate) buffer solution adjusted to pH 7.5. Solution was delivered in a volume of 10 ml/kg. The second group of animals received IP injections of vehicle alone. Free access to standard laboratory food and water was provided for both groups on a *continuing basis.*

Testing for hyperglycemia was conducted 1 week following injection of STZ. All rats assigned to the diabetic group were found to be hyperglycemic (blood-glucose >25 mmol/l) through the use of a "Glucoscan" (Model #3000, Lifescan Inc., Mountain View, CA) and a tail pick blood sample.

Experiment 1

After a 4-week period of STZ-induced diabetes mellitus, behavioral observations were initiated on animals. The experimental population was randomly divided into two groups with half the diabetics and half the normals in each group. Each group was then tested on alternating nights.

Rats were transported from the housing facility to the experimental room after weighing. After transport, study animals were placed one at a time in the observational test cage (26 × 18 × 18 cm). At the end of a 10-min habituation period, each rat was injected with a pseudorandomly chosen, preassigned dose of APO. Apomorphine was dissolved in a solution of phosphate-buffered normal saline (pH 7.4) and ascorbic acid (0.5 mg/kg). Administrations of APO and vehicle were given SC in the loose skin on the back of the neck. Each rat received each dose (vehicle, 20, 40, 80, and 120 μg/kg: 5 ml/kg) only once. Animals were monitored in real time by using a videocamera (VK-C 1500, Hitachi Ltd., Tokyo, Japan) connected to a monitor (MT-2860, Hitachi) in an adjacent room. The videocamera was placed underneath and to the side of the test cage to obtain the best view of the animal's behavior. Each animal was observed for a 30-min period in which the number of yawns was counted and tabulated by the experimenter. A yawn was identified as an apparently involuntary wide opening of the mouth accompanied by an apparent respiratory movement but not associated with functional mastication. Experimentation began at 1900 h and each rat was tested at the same time of day to minimize variation due to circadian rhythm. At the completion of behavioral observations, rats were retested for hyperglycemia. Only those rats with blood-glucose levels equal to or exceeding 25 mmol/l were included in the data analysis. Experiment 1 results were analyzed using a between-groups analysis of variance (ANOVA) to compare APO-stimulated yawning behavior in normal and 4-week-diabetic rats.

Experiment 2

Seven rats injected with STZ (from Experiment 1) to produce diabetes mellitus were used in Experiment 2. Housing, experimental conditions, and hyperglycemic testing were identical to Experiment 1 except only four doses of APO (20, 40, 80, and 120 μg/kg) were used. Behavioral observations originally initiated at 4 weeks of untreated diabetes were repeated at weeks 8 and 20. Only those rats that endured the entire 20 weeks were included in data analyses; again a blood-glucose level of >25 mmol/l was the minimum level required for animals to be considered diabetic. Results were analyzed using a within-groups ANOVA to compare yawning behavior during the progression of STZ-induced diabetes mellitus. Number of yawns in 8- and 20-week-diabetic rats is compared with the number of yawns observed at 4 weeks of diabetes.

RESULTS

Apomorphine, in doses sufficient to stimulate presynaptic dopaminergic receptors, has a reduced ability to initiate yawning in short-term (4 weeks) diabetic rats as compared with their age-matched controls. Although injections of APO in the range 20–120 μg/kg increased yawning behavior above saline control in both normal and diabetic rats, Fig. 1 shows that the mean number of yawns is less in STZ-treated rats.

Yawning behavior was significantly greater in normal rats as compared with the diabetic group after 20 μg/kg administration of APO $F(1, 14) = 11.90$, $p < 0.01$, 40 μg/kg, $F(1, 14) = 70.39$, $p < 0.001$, and 80 μg/kg, $F(1, 14) = 7.67$, $p < 0.05$. At 120 μg/kg, the difference in yawning rate failed to reach significance.

In Experiment 2, a within-groups ANOVA revealed no significant differences in yawning behavior between the 4- and 8-week observational periods at any of the four doses of APO tested. Conversely, when rats of 4-week duration of untreated diabetes were compared with those of 20-week duration an increase in the number of yawns (demonstrating recovery) was seen at several doses (Fig. 2). Administration of APO dose 20 μg/kg produced significantly more, $F(1, 6) = 14.06$, $p < 0.01$, yawns in rats after 20 weeks of diabetes than those rats tested earlier at only 4-week duration of hyperglycemia. At APO dose 40 μg/kg, no significant differences were seen; however, at APO dose 80 μg/kg 20-week-diabetic rats again yawned significantly more, $F(1, 6) = 8.11$, $p < 0.05$, than they had 16 weeks earlier at 4 weeks' diabetes mellitus. The differences seen at APO dose 120 μg/kg did not reach significance.

t-tests showed that at any of the APO doses tested yawning was not significantly different between normal control rats and 20-week-diabetic rats.

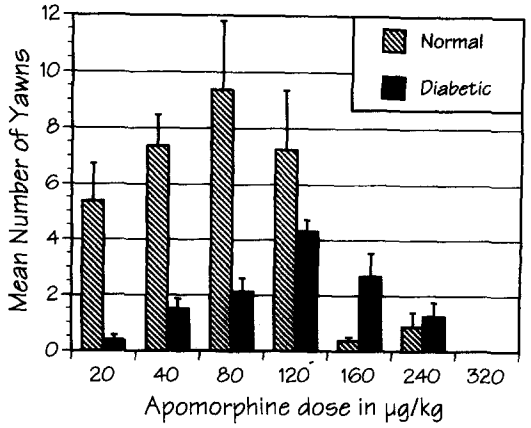

FIG. 1. Mean yawning behavior (±SE) at each dose of apomorphine for normal and 4-week-diabetic rats.

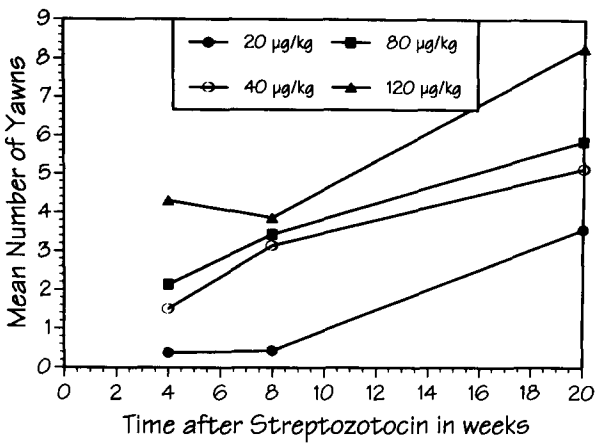

FIG. 2. Changes in mean yawning behavior (+SE) per 30-min observation period at each dose of apomorphine as a function of duration of diabetes.

DISCUSSION

The results show that experimental STZ-induced diabetes mellitus of relatively short duration (4 weeks) produces a decreased ability of APO to stimulate yawning behavior in rats. As the duration of diabetes progresses, a recovery in the ability of APO to induce yawning behavior was observed. Although animals appeared systemically more sick (diarrhea, coat condition, cataracts) as duration of diabetes progressed, yawning behavior was increased above the level seen in 4-week-diabetic rats and was not significantly different from that seen in normal rats at any of the APO doses tested. It is possible that as animals adapt to the hyperglycemic state alterations in the neurochemical milieu responsible for the initially decreased yawning behavior are restored or compensatory physiological mechanisms are then activated.

Although alterations in yawning behavior in humans have been noted in diseases known to affect the DA system, such as Parkinson's disease, schizophrenia, and Huntington's chorea (11), we are not aware of any data suggesting an alternation during diabetes mellitus. However, the results of the present study show that a malfunction in yawning behavior does occur in an animal model of chemically produced diabetes mellitus (at least in the short term). The present study clearly identified the ability of a pathologic process such as diabetes mellitus to alter the necessary milieu responsible for APO-induced yawning behavior.

Although the precise circuitry involved in the production of APO-induced yawning has not yet been identified, it is clear that yawning behavior seen as a result of APO stimulation is due to central processes and is not the result of peripheral stimulation. For example, lesions of the paraventricular nucleus of the hypothalamus abolish yawning behaviour (1) while direct application of APO (13) or oxytocin (12) to the paraventricular nucleus stimulate yawning behavior in rats. These substances produce no significant effect on yawning when injected into the caudate nucleus, preoptic area, or ventromedial nucleus. In addition, while centrally acting DA antagonists such as metoclopramide, sulpiride, and haloperidol diminish yawning behavior domperidone, a peripherally acting DA antagonist that does not readily cross the blood-brain barrier (10), does not produce any diminution in APO-stimulated yawning (16).

Further experimental work is needed to determine the precise pathway(s) and cascade of neurochemical events responsible for the induction of dopaminergically mediated yawning. However, as the elucidation of the specific mechanisms necessary for the stimulation of yawning behavior become available these may provide significant insight into diabetes mellitus and its manifestations as a result of alterations in the CNS. An alteration in the neurochemical milieu as a function of diabetes mellitus may contribute to the manifestations of the disease and an increased focus directed toward the establishment of these changes occurring within the CNS should be carried out.

ACKNOWLEDGEMENTS

The authors acknowledge the support of the Canadian Diabetes Association and the Pharmaceutical Manufacturers Association of Canada and the Medical Research Council of Canada.

REFERENCES

1. Argiolas, A.; Melis, A.; Mauri, A.; Gessa, G.: Paraventricular nucleus lesion prevents yawning and penile erection induced by apomorphine and oxytocin but not by ACTH in rats. Brain Res. 421:349-352; 1987.
2. Argiolas, A.; Melis, M.; Gessa, G. Calcium channel inhibitors prevent apomorphine- and oxytocin-induced penile erection in male rats. Eur. J. Pharmacol. 166:515-518; 1989.
3. Argiolas, A.; Melis, M.; Vargiu, L.; Gessa, G. d(CH_2)$_5$Tyr(Me)-Orn^8-vasotocin, a potent oxytocin antagonist, antagonizes penile erection and yawning induced by oxytocin and apomorphine, but not by ACTH(1-24). Eur. J. Pharmacol. 134:221-224; 1987.
4. Drukarch, B.; Stoof, J. D_2 dopamine autoreceptor selective drugs: Do they really exist? Life Sci. 47:361-376; 1990.
5. Gower, A.; Berendsen, H.; Princen, M.; Broekkamp, C. The yawning penile erection syndrome as a model for putative dopamine autoreceptor activity. Eur. J. Pharmacol. 103:81-89; 1984.
6. Heaton, J.; Varrin, S. Metoclopramide decreases apomorphine-induced yawning and penile erection. Pharmacol. Biochem. Behav. 38:917-920; 1991.
7. Heaton, J.; Varrin, S. Studies on the diabetic rat as a viable model of impotence. Int. J. Impotence Res. 2(2):117-124; 1991.
8. Heaton, J.; Varrin, S. The impact of alcohol ingestion on erections in rats as measured by a novel bioassay. J. Urol, 145:192-194; 1991.
9. Heaton, J. P. W.; Varrin, S.; Morales, A. The characterization of a bioassay for rat erectile function. J. Urol. 145:1099-1102; 1991.
10. Laduron, P.; leysen, J. Domperidone, a specific in vitro dopamine antagonist, devoid of in vivo central dopaminergic activity. Biochem. Pharmacol. 28:2161-2165; 1979.
11. Lehman, H. Yawning. A homeostatic reflex and its psychological significance. Bull. Menninger Clin. 43:123-126; 1979.
12. Melis, M.; Argiolas, A.; Gessa, G. Oxytocin-induced penile erection and yawning: Site of action in the brain. Brain Res. 398:259-265; 1986.
13. Melis, M.; Argiolas, A.; Gessa, G. Apomorphine-induced penile erection and yawning: Site of action in the brain. Brain Res. 415: 98-102; 1987.
14. Neubauer, B.; Christensen, N. Norepinephrine, epinephrine and dopamine contents of the cardiovascular system in long-term diabetics. Diabetes 25:6-10; 1976.
15. Okuyama, S.; Hashimoto, S.; Aihara, H. Effects on the caudate

spindle in rats of dopamine microinjected into the caudate nucleus. Neurosci. lett. 59:27–32; 1985.
16. Pehek, E.; Thompson, J.; Eaton, R.; Bazzett, T.; Hull, E. Apomorphine and haloperidol, but not domperidone, affect penile reflexes in rats. Pharmacol. Biochem. Behav. 31:201–208; 1988.
17. Saller, C. F. Dopaminergic activity is reduced in diabetic rats. Neurosci. lett. 49:301–306; 1984.
18. Skirboll, L. R.; Grace, A.; Bunney, B. Dopamine auto- and postsynaptic receptors: Electrophysiological evidence for differential sensitivity to dopamine agonists. Science 206:80–82; 1979.
19. Stahle, L.; Ungerstedt, U. Yawning and suppression of exploration in amphetamine treated rats: Incompatibility with the autoreceptor hypothesis. Psychopharmacology (Berl.) 97:275–276; 1989.
20. Stoessl, A.; Dourish, C.; Iversen, S. Apomorphine-induced yawning in rats is abolished by bilateral 6-hydroxydopamine lesions of the substantia nigra. Psychopharmacology (Berl.) 93: 336–342; 1987.
21. Trulson, M. E.; Hinmel, C. D. Decreased brain dopamine synthesis rate and increased [^{3}H]spiroperidol binding in streptozotocin-diabetic rats. J. Neurochem. 40:1456–1459; 1983.
22. Yamada, K.; Furukawa, T. Direct evidence for involvement of dopaminergic inhibition and cholinergic activation in yawning. Psychopharmacology (Berl.) 67:39–43; 1980.