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Increased S(−)-Apomorphine–Induced Vacuous Chewing and Attenuated Effect of Chronic Haloperidol Treatment in Streptozotocin-Induced Diabetic Rat

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SUMIYOSHI, T., J. ICHIKAWA AND H. Y. MELTZER. *Increased S(−)-apomorphine–induced vacuous chewing and attenuated effect of chronic haloperidol treatment in streptozotocin-induced diabetic rat.* PHARMACOL BIOCHEM BEHAV **57** (1/2) 19–22, 1997.—The incidence of S(−)-apomorphine–induced vacuous chewing movements (VCMs) as a model for tardive dyskinesia was investigated in streptozotocin (STZ)-induced diabetic rats. A single dose of STZ (65 mg/kg, intravenously) caused a diabetic state (hyperglycemia, 480–490 vs. 116–118 mg/dl in naive rats). S(−)-apomorphine (250 μg/kg, subcutaneously)–induced VCMs were significantly intensified in diabetic rats which had received STZ 9 weeks previously. The enhancement of VCMs was also observed in nondiabetic rats which received subsequent treatment with depot haloperidol (4 mg/kg, intramuscularly, once a week, every week for 4 weeks) followed by a 2-week washout period. The ability of haloperidol to enhance VCMs was attenuated in diabetic rats. The implications of these results in relation to altered neurotransmissions in STZ-induced diabetes are discussed. © 1997 Elsevier Science Inc.

Diabetes Streptozotocin Vacuous chewing movements Haloperidol Dopamine receptors Tardive dyskinesia

A higher incidence of spontaneous dyskinesias has been reported in patients with diabetes mellitus (DM) (7). An increased incidence of tardive dyskinesia (TD) associated with chronic neuroleptic treatment in patients with schizophrenia who are comorbid for DM has been noted (7,17,32). Abnormalities in dopaminergic function have been suggested to play a role in the pathophysiology of TD by some (11,13,14,18) but not all (24,30) investigators. Specifically, supersensitivity of some types of striatal dopamine (DA) receptors (e.g., D_1 and D_2) after chronic blockade by neuroleptic treatment has been suggested to be the basis for TD (11,26). DA may also be involved in the increased risk of TD in DM. Thus, streptozotocin (STZ)-induced DM rats have decreased DA synthesis and turnover in various brain regions, including the striatum (1,2,22,29). STZ- or alloxan-induced DM rats also have increased DA-D_2 (15,29) and decreased D_1 receptors (21) in the striatum. Apomorphine (a nonselective D_1/D_2 agonist)-induced vacuous chewing movements (VCMs) (31) or stereotyped behaviour (27) have been described in rats. An increased incidence of apomorphine-induced or spontaneous VCMs following chronic treatment with neuroleptics such as fluphenazine and haloperidol (HPD) have been reported in studies using animal models of TD (24, 26,30,31). A former study (31) has suggested that apomorphine-induced VCMs and other stereotypies in rats have distinct substrates which are differentially influenced by prolonged neuroleptic treatment. Apomorphine-induced VCMs were enhanced following chronic neuroleptic treatment when other stereotypies were suppressed in that study (31). In view of the increased risk of TD in DM patients, it is hypothesized that rats with experimental DM would show an increased incidence of VCMs.

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TABLE 1

CHANGE IN RAT BODY WEIGHT

Time After STZ Injection (wk)	Treatment			
	Vehicle–Vehicle (n = 10)	Vehicle–HPD (n = 9)	STZ–Vehicle (n = 12)	STZ–HPD (n = 12)
0	257	258	247	287
1	360	322	278	305
2	450	390	291	303
3	476	441	279	322
4	511	476	259	316
5	538	521	265	309
6	569	549	285	324
7	598	579	272	300
8	620	600	270	322
9	641	620	264	322

Values represent average body weight (g) for each group.

The present study investigated the effect of STZ-induced DM on $S(-)$-apomorphine–induced VCMs in rats in relation to chronic HPD treatment.

METHOD

Male Sprague–Dawley rats (Zivic Miller Laboratories, Pittsburgh, PA), weighing 200–250 g on arrival in the laboratory, were used. The rats were housed in groups of five or six in standard floor net cages in a light- and temperature-controlled room and had free access to food and water.

Animals were rendered diabetic according to an established protocol (2). STZ (65 mg/kg, in 0.01 M citrate buffer, pH 4.5, 1 ml/kg; Sigma Chemical Co., St. Louis, MO) was injected into the lateral tail vein. An intravenous dose of 65 mg/kg STZ was chosen based on previous studies investigating neurochemical changes in brain DA, 5-HT, and other neurotransmitters (2,3,12). Testing for hyperglycemia was conducted 1 week following injection of STZ through the use of a Glucometer Encore (Model no. 5885A; Miles Inc., Elkhart, IN) and tail-pick blood samples. the body weight of rats was measured every week following the STZ injection.

Four weeks following STZ treatment, both DM and non-DM rats were allocated into HPD-treated groups (STZ–HPD or vehicle–HPD group) or vehicle-treated groups (STZ–vehicle or vehicle–vehicle group). HPD-treated groups received intramuscular (IM) injection of the long-acting depot HPD (Decanoate; McNeil) 4 mg/kg, once a week for 4 weeks. Vehicle groups received sesame oil. With this protocol for HPD treatment, a significant increase in the density of the striatal D_2 receptors (31%) has been observed in non-DM rats (unpublished data) in accord with a previous study (6).

The number of VCMs in rats was counted 14 days after the final depot HPD injection. Rating sessions were held from 1300 to 1500 h in a soundproof room. In the morning of the day of observation, rats were transferred from their home cages to standard plastic cages followed by about 4 h of habituation periods. All ratings were performed by a well-trained rater blinded to treatment. VCMs were measured for 90 min in 15-min intervals following the injection of $S(-)$-apomorphine [250 μg/kg, subcutaneously (SC); Research Biochemicals, South Natick, MA] dissolved in physiologic saline with 100 μM ascorbic acid. Only nonstereotyped VCMs (in bouts), consisting of rapid chewing movements of the lower jaw, which were not directed toward specific objects were counted (4,31). The movements were separated into those that contained a burst of facial tremor and those that did not, as described in a former study (4). Only purposeless chewing movements not associated with facial tremor (4) were measured. Chewing movements related to sniffing, yawning, tongue protrusions, grooming, gnawing, mouthing food, or penile grooming were observed but not counted.

The sum of the number of VCMs during the 90-min observation period for each treatment group was compared by analysis of variance (ANOVA) followed by posthoc comparisons (Scheffé's test). A p value < 0.05 was considered significant.

TABLE 2

BLOOD GLUCOSE LEVELS IN RATS

Treatment (n)	Glucose Levels (mg/dl)
Vehicle–vehicle (10	116.1 ± 4.6
Vehicle–HPD (9)	117.8 ± 4.8
STZ–vehicle (12)	480.4 ± 16.8
STZ–HPD (12)	490.4 ± 17.3

Values represent mean ± SEM.

RESULTS

STZ-treated rats showed a loss of weight gain which continued throughout the study period (Table 1). Blood glucose levels in STZ-induced DM rats 1 week after STZ treatment (STZ–HPD and STZ–vehicle groups) were elevated about fourfold compared with non-DM rats (vehicle–HPD and vehicle–vehicle groups) (Table 2). All rats assigned to the DM groups (STZ–HPD and STZ–vehicle) were found to be hyperglycemic (blood glucose $>$ 332 mg/dl). Diabetes was also verified by glucosuria (Chemstrip; Boehringer Mannheim Co.).

Figure 1 shows the sum of the number of VCMs during the 90-min observation period for each treatment group. A significant group difference was noted [$F(3, 39) = 29.58$, $p < 0.0001$]. Posthoc analyses revealed significantly larger numbers in VCMs for the vehicle–HPD, STZ–vehicle, and STZ–HPD groups compared with the vehicle–vehicle group ($p < 0.01$). The numbers of VCMs for the STZ–vehicle and STZ–HPD groups were significantly smaller than that for the vehicle–HPD group ($p < 0.001$). The number of $S(-)$-apomorphine–induced

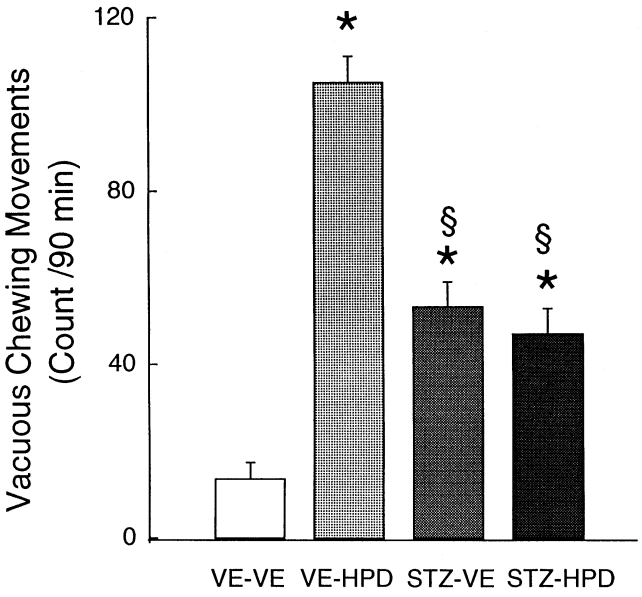

FIG. 1. Effects of streptozotocin (STZ; 65 mg/kg, IV), depot haloperidol (HPD; 4 mg/kg per week, IM × 4 weeks) or combined treatment thereof on S(−)-apomorphine (250 μg/kg, SC)–induced vacuous chewing movements in rat. Values represent mean ± SE. VE-VE, vehicle–vehicle group ($n = 10$); VE-HPD, vehicle–haloperidol group ($n = 9$); STZ-VE, streptozotocin–vehicle group ($n = 12$); STZ-HPD, streptozotocin–haloperidol group ($n = 12$). *$p < 0.01$, significant difference from vehicle–vehicle group. §$p < 0.001$, significant difference from vehicle–HPD group.

VCMs for the STZ–HPD group was not significantly different from that of the STZ–vehicle group ($p = 0.83$).

DISCUSSION

The present study provides the first evidence for enhancement of S(−)-apomorphine–induced VCMs in STZ-induced DM rats. Moreover, the results confirm the previous finding of an increased incidence of apomorphine-induced VCMs following chronic neuroleptic treatment (31). The present findings also demonstrated a lower incidence of S(−)-apomorphine–induced VCMs in rats receiving both HPD and STZ relative to animals receiving HPD alone.

The present study used 250 μg/kg S(−)-apomorphine, a dose which induces other stereotyped behaviors (i.e., sniffing, licking) in rats (8,10) in addition to VCMs. Therefore, it is possible that in the present study, VCMs were partly masked by concurrent stereotyped behaviour (i.e., sniffing, licking). Investigation of VCMs with various doses of apomorphine should further clarify the increase in VCMs in DM rats.

The precise mechanism for the increase in S(−)-apomorphine–induced VCMs in STZ-induced DM rats is currently unclear. One of the possibilities is that the altered dopaminergic activity reported in DM (1,2,22,29) is responsible for the increased VCMs, because apomorphine-induced or spontaneous VCMs have been observed following chronic treatment with neuroleptics which are DA receptor blockers (24,26,30,31). Chronic treatment with HPD, a D_2 receptor antagonist (23), also caused an enhancement of VCMs induced by S(−)-apomorphine in non-DM rats in the present study, in accordance with a previous report which used fluphenazine (31). Therefore, some functional changes in D_2 and/or D_1 receptors may be responsible for the enhanced S(−)-apomorphine–induced VCMs in STZ-induced DM rats. It has been demonstrated that blockade of D_1 receptors attenuates neuroleptic-induced DAergic supersensitivity (5) and that stimulation of D_1 receptors is necessary to elicit apomorphine-induced stereotypies (14). These findings appear to contradict the present results, as the D_1 receptor density has been reported to decrease in DM rats (21). However, because S(−)-apomorphine is a nonselective D_1/D_2 agonist, the effect of altered D_2 receptor–mediated neurotransmission on VCMs in DM rats should also be considered. The previous study (21) evaluated the D_1 receptor density in rat brain only 4 weeks after induction of DM, whereas the current study measured S(−)-apomorphine–induced VCMs in rats more than 9 weeks after STZ treatment. The difference in the experimental designs may be another reason for the discrepant results as to the changes in the D_1 receptor–related neurotransmissions in DM rats. A number of manipulations on other neurotransmitter systems including the cholinergic system (9,20) have been reported to affect the incidence of VCMs. Therefore, involvement of other neurotransmitters in addition to DA should also be considered to explain increased VCMs in DM rats.

Recent studies have suggested the involvement of GABAergic dysfunction in HPD-induced VCMs (24,25). Decreased GABA concentrations have been reported in the hypothalamus and brain stem of rats treated with STZ 3 weeks before death [40 mg/kg, intravenously (IV)] (28). Altered GABAergic activity in STZ-induced diabetic rats may explain the underlying mechanism for the enhancement of S(−)-apomorphine–induced VCMs observed in the present study.

Hydroxy radical formation in the brain has been suggested to be significant for the development of TD (8,18). A recent study has reported that formation of hydroxy radicals is facilitated in the brain of STZ-induced DM rats owing to the hyperglycemic effect of STZ (19). Thus, increased hydroxy radical formation in the brain may be another possible basis for the enhancement of S(−)-apomorphine–induced VCMs in STZ-induced DM rats.

Chronic treatment with HPD in STZ-induced DM rats did not affect S(−)-apomorphine–induced VCMs. As it is possible from the present results that STZ-induced DM could cause functional changes in DA receptors, it can be speculated that HPD treatment has little effect on DA (D_2 and/or D_1) receptor sensitivity following STZ-induced DM. Other possibilities may include decreased availability of HPD in the brain of STZ-induced DM rats, as it has been reported that cerebral blood flow is altered in animals with DM [see (16) for review]. Thus, the availability of HPD in the brain following STZ-treatment requires further study.

The present results also suggest that pretreatment with STZ attenuates the ability of HPD to increase S(−)-apomorphine–induced VCMs. As mentioned above, the possible functional changes in DA receptors and/or altered pharmacokinetics of HPD may be responsible for the decreased S(−)-apomorphine–induced VCMs in STZ-induced DM rats. Another explanation is that the combined treatment of STZ and HPD may, in fact, enhance the sensitivity of DA receptors to S(−)-apomorphine relative to HPD treatment alone. If this were the case, then STZ-induced DM may produce an increase in S(−)-apomorphine–induced stereotypy, which may interfere with expression of S(−)-apomorphine–induced VCMs. Further studies which investigate correlations between the incidence of S(−)-apomorphine–induced VCMs and that of stereotypies in DM rats are expected to resolve this issue.

In conclusion, the present study has demonstrated enhance-

ment of *S*(−)-apomorphine–induced VCMs in STZ-induced DM rats, as well as in non-DM rats which received chronic HPD treatment. The effect of HPD on *S*(−)-apomorphine–induced VCMs was attenuated in STZ-induced DM rats. Further studies (e.g., measurement of stereotypies) would help determine whether the present findings in DM rats are relevant to the increased risk of TD in patients with DM who receive neuroleptic treatment.

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