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Reduction of Nigral Glutamic Acid Decarboxylase in Rats with Neuroleptic-Induced Oral Dyskinesia

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Abstract. Following eight monthly haloperidol decanoate injections rats showed an increased rate of vacuous chewing movements (VCM's), which gradually disappeared within 4 drug-free months. Another single dose of non-decanoate haloperidol reinstated a second increase in VCM rate which was still significant after 2 months. The glutamic acid decarboxylase (GAD) activity in the substantia nigra of these chronically haloperidol-treated rats was lower than untreated controls. Furthermore, there was a significant negative correlation between individual VCM rates and nigral GAD activity. No corresponding changes occurred in other brain regions. The depression of nigral GAD may reflect a reduced tissue density of GABA-ergic axon terminals within the descending striato-nigral pathway.

Key words: Oral dyskinesia – Chronic neuroleptic – Vacuous chewing movements – Movement disorder – Tardive dyskinesia – Nigral glutamic acid decarboxylase – Striato-nigral GABA-ergic system – Rat

Long-term administration of neuroleptic drugs has been reported to induce a long-standing fairly regular vacuous chewing behavior in rats with certain brain lesions (Glassman and Glassman 1980; Gunne et al. 1982). A similar effect was occasionally observed also in unlesioned rats after 12 weeks of haloperidol decanoate administration. The possible relationship between these phenomena and neuroleptic-induced tardive dyskinesia in humans has been discussed (Gunne et al. 1982).

Various attempts have been made to explain movement disorders resulting from chronic neuroleptic treatment as due to dopamine receptor supersensitivity (Carlsson 1970; Klawans 1973; Tarsy and Baldessarini 1977). During recent studies on a primate model for tardive dyskinesia (Barany et al. 1979) our interest was focussed on changes within the GABA-ergic rather than the dopaminergic system. Long-term administration of classical neuroleptics has been reported to induce a typical series of GABA-related changes in the substantia nigra. The turn-over rate of GABA is decreased (Mao et al. 1977), there is an enhanced binding of ^{3}H-GABA (Gale 1980) and a rise in GABA receptor sensitivity in this area (Scheel-Krüger et al. 1981).

The present paper shows a relationship between neuroleptic-induced vacuous chewing behavior in rats and a depression of the GABA synthesizing enzyme L-glutamic acid decarboxylase (GAD) in the substantia nigra. These observations seem to link neuroleptic-induced oral dyskinesias to a reduction of nigral GABA-ergic function.

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Materials and Methods

Subjects, Treatment and Procedure. Twenty-four female albino rats (Sprague Dawley, Anticimex Ltd, Sollentuna) initially weighing 170 – 180 g were housed in single cages with a 12-h light-dark cycle, room temperature of 20° C, and with free access to tap water and food (rat pellets). Seventeen animals received haloperidol decanoate 7.1 mg/rat IM once monthly for 8 months, corresponding to approximately 1 mg/kg/day of haloperidol, whereas seven were untreated controls. This treatment period was followed by 5 months without injections, after which each rat received a single dose of non-decanoate haloperidol (1 mg/kg IP). The rats were observed monthly for vacuous chewing movements (VCM's) before each haloperidol decanoate administration. When the oral dyskinesias became prominent, observations were also made at various intervals between the injections. Observations of individual rats were made in a $2 \times 3 \times 4$ dm Plexiglas cage with a mirror behind it and counting of mouth movements was carried out according to Gunne et al. (1982). Three haloperidol-treated rats died during the experiment for reasons unrelated to drug treatment (two were drowned due to a faulty drinking water valve, one had a neck tumor). Otherwise the animals appeared to be in good shape throughout the experiment.

Sixty-seven days after the last dose of haloperidol, the rats were killed by decapitation and the brains immediately dissected. The brains were cooled for 10 min in a −30° C freezer to facilitate sectioning. Two 1 mm coronal slices were cut: one 2 mm rostral to the optic decussation, the other immediately in front of the pons. From the first of these sections striatal tissue and accumbens were dissected, and from the second the nigral region (below the medial lemnisci) and the superior colliculus region (the division was made horizontally half way down, between the upper surface of the mesencephalon and the aqueduct). In addition 10 – 15 mg samples were taken from the frontal cortex. Left and right sections were analysed separately for GAD activity, but all values were expressed as means of the two sides. The mean difference between the sides was 10.4% (range 0 – 19.6%). The tissue samples were kept frozen at −80° C until analysis.

Measurement of GAD Activity. GAD activity was measured by trapping the $^{14}CO_2$ formed from carboxyl-labelled gluta-

mic acid. The method is a modification of Wu et al. (1976) and Urquhart et al. (1975). Brain tissue was homogenized in 500 µl of 0.1 M potassium phosphate buffer pH 6.5, containing 0.1 mM pyridoxal phosphate, 1 mM 2-aminoethylisothiouroniumbromide and 0.25% Triton X-100. A 350 µl sample was incubated for 30 min at 37° C with 250 µl of 0.1 M potassium phosphate buffer pH 7.5 containing 0.1 mM pyridoxal phosphate, 4 mM sodium arsenite, 0.8 M L-glutamic acid and 0.5 µCi L-[1-^{14}C]-glutamic acid (New England Nuclear, Southampton, UK). The incubation was terminated by adding 0.1 ml of 4 M sulphuric acid, and the CO_2 formed was collected in a CO_2-trapping apparatus into 1 ml of tissue solubilizer (TS-1, Koch-Light Laboratories Ltd, Haverhill, UK). After 18 h at 4° C the activity was measured in a liquid scintillation spectrometer.

Statistical Methods. For statistical purposes 5 of the 21 observation days were selected (day 1 = baseline level; day 210 = peak in VCM frequency during haloperidol-decanoate; day 360 = decline in VCM 5 months after the final haloperidol-decanoate dose; day 374 = peak in VCM after a single injection of non-decanoate haloperidol and day 420 = last measurement of VCM). VCM's in the haloperidol-decanoate treated rats and untreated controls were compared between groups on these five occasions by a two-way analysis of variance (split-plot, least square design). Subsequent to this, two comparisons were made between VCM means for days 1 and 210, 360 and 420 using the Scheffe ratio (Kirk 1968).

Group means were compared using independent Student's *t*-tests. The degree of association between GAD activity and VCM rate was tested by computing the product-moment correlation coefficient.

Results

After 4 months of treatment with haloperidol decanoate some rats began to show mouth movements (Fig. 1). These mostly consisted of quick single mouth openings with only vertical movements of the jaw. Occasionally clusters of three or more jaw movements occurred, often preceded by short muscle twitching in the masseteric area. The frequency of these VCM's increased as the administration continued. Table 1 shows the VCM rates in untreated controls and haloperidol treated rats at different time points during the experiment. Two way ANOVA yielded a significant group effect [$F(1, 19) = 12.00$, $P < 0.01$] showing that during the whole 14 months experiment the haloperidol-treated rats had a higher VCM frequency than untreated controls. Furthermore, there were significant *F*-values for the time factor [$F(4,76) = 15.04$, $P < 0.01$] as well as the interaction factor [$F(4,76) = 7.44$, $P < 0.01$] verifying that the haloperidol-treated rats showed an increase in VCM frequency over time, while the control group remained at the baseline level throughout the observation period. Comparison between baseline frequency of VCM and the peak level after eight monthly haloperidol decanoate injections showed a significant elevation [$F = 22.28$, $P < 0.01$]. When haloperidol decanoate was discontinued after eight monthly injections the frequency gradually declined, and 5 months later only sporadic oral movements in some rats could be seen. A single injection of non-decanoate haloperidol 1 mg/kg then produced another increase in VCM frequency. When a comparison was made between VCM frequency before and after the single final dose of non-decanoate haloperidol the difference was found to be significant [$F = 58.92$, $P < 0.01$].

The GAD activity remained unchanged in all areas investigated, except in the substantia nigra (Table 2) where the level was significantly lower in the rats which received haloperidol compared to untreated controls. Among the haloperidol-treated rats there was a significant negative correlation ($P < 0.005$) between the frequency of mouth movements and GAD activity in the substantia nigra (Fig. 2). No such correlation could be seen in the striatum, accumbens, superior colliculus or frontal cortex.

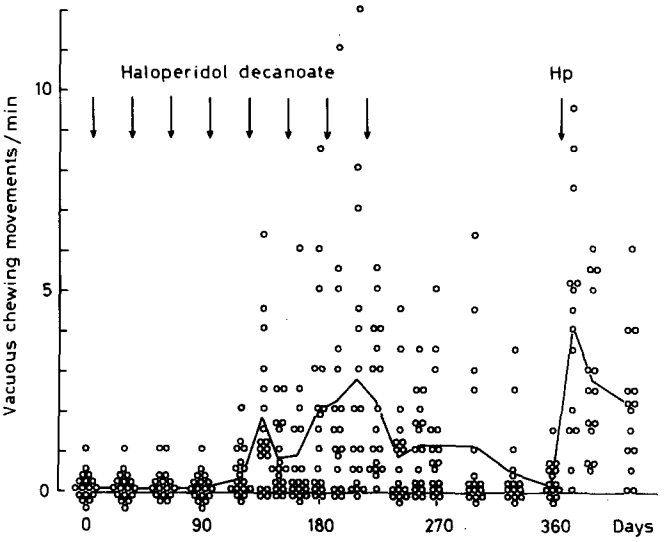

Fig. 1. Vacuous chewing movements mean (*curve*) and individual levels (*circles*) in 17 rats during administration of haloperidol decanoate followed by a final injection of non-decanoate haloperidol (*Hp*) at time points indicated by *arrows*

Table 1. Vacuous chewing movements per min (means ± SD) in rats given chronic haloperidol treatment and untreated controls at five time points during a 14-month experiment. Number of animals within brackets

Day	Untreated controls	Chronic haloperidol	Significance
0	0.2 ± 0.3 (7)	0.1 ± 0.2 (17)	N.S.
210	0.1 ± 0.2 (7)	2.7 ± 3.5 (17)	N.S.
360	0.1 ± 0.2 (7)	0.3 ± 0.5 (14)	N.S.
374	0.2 ± 0.3 (7)	4.2 ± 2.9 (14)	$P < 0.01$
420	0.1 ± 0.2 (7)	2.1 ± 1.7 (14)	$P < 0.05$

Table 2. GAD activity (µkatal/dm^3) in different brain areas of rats given chronic haloperidol treatment for 14 months and untreated controls (means ± SD)

Area	Untreated controls ($n = 7$)	Chronic haloperidol ($n = 14$)	Significance
Frontal cortex	9.9 ± 1.6	9.0 ± 1.3	N.S.
Striatum	7.8 ± 1.3	10.8 ± 4.1	N.S.
Accumbens	18.6 ± 5.4	19.8 ± 5.1	N.S.
Sup. colliculus	18.5 ± 5.2	21.0 ± 5.5	N.S.
Subst. nigra	27.5 ± 5.3	18.7 ± 2.8	$P < 0.001$

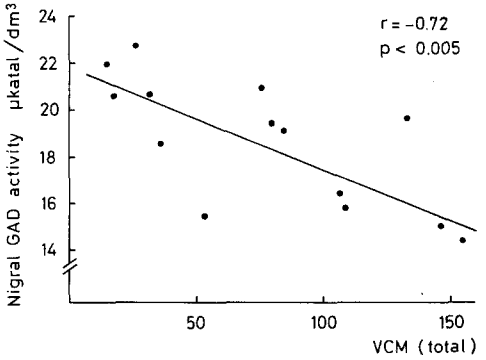

Fig. 2. Linear regression and individual values for nigral GAD activity plotted against total (cumulative) number of vacuous chewing movements recorded in rats at 21 observational 2 min sessions during a 14 months' experiment

Discussion

The present study has demonstrated a negative correlation between a neuroleptic-induced movement disorder in the oral region and GAD activity in the substantia nigra. Gunne et al. (1982) have reported that frontal cortex ablation produced similar oral dyskinesias in rats as did chronic haloperidol administration (the combination of both gave higher chewing rates than either procedure alone). In this context it appears noteworthy that Scatton et al. (1982) found lowered nigral GAD activities in rats which had been submitted to prefrontal cortex ablation. This specific brain lesion thus represents another example in which chewing behavior develops together with low nigral GAD. In our studies on a primate model of tardive dyskinesia we have presented preliminary evidence for a corresponding reduction of GAD activity in the substantia nigra of *Cebus apella* monkeys made dyskinetic by long-term administration of haloperidol (Häggström et al. 1981). On the other hand Lloyd and Hornykiewicz (1977) found no changes in nigral GAD activity when haloperidol had been given chronically to rats for 167 days. After a corresponding length of haloperidol administration we had only noticed a beginning rise in VCM's, but unfortunately we have no measurements of GAD activity at that time point in the present experiments.

As mentioned above, there is evidence that long-term administration of classical neuroleptic drugs such as haloperidol has a profound effect on GABA-ergic function within the substantia nigra. The turn-over rate of GABA goes down (Mao and Costa 1978), whereas postsynaptic GABA receptors increase their binding capacity (Gale 1980) and become supersensitive (Scheel-Krüger et al. 1981). Together these findings seem to indicate a major functional reduction in activity within the striato-nigral GABA pathway. This neuroleptic effect is probably mediated via disinhibited striatal GABA-ergic interneurones situated between the dopaminergic terminals and the striato-nigral GABA system (Gale and Casu 1981).

A correct interpretation of the dyskinesia-related nigral GAD reduction may have to await further studies, including an immunohistochemical investigation of possible changes within GABA-ergic nerve terminals. Immunocytochemically defined GAD is preferentially located in axon terminals of GABA-ergic neurones (Ribak et al. 1979), and a reduction of brain GAD has been interpreted to reflect a selective loss of GABA neurones, for instance in Huntingtons chorea (Bird and Iversen 1974; McGeer and McGeer 1976). Itoh and Uchimura (1981) and Itoh (1983) found a reduced GAD holoenzyme activity in the rat substantia nigra reticulata after 10 days of haloperidol administration. In their study the addition of pyridoxal-5-phosphate had been omitted in order to obtain a measurement of the true neuronal rate of GABA formation, which appears to be cofactor regulated. The GAD activity recorded in the present paper, with added cofactor, probably reflects the brain tissue GABA-ergic terminal density rather than current neuronal enzyme activity level.

Two months after the final injection of regular (non-decanoate) haloperidol there was still a depression of GAD activity in the substantia nigra. This considerable duration indicates that the long-term neuroleptic-induced depression may be irreversible. It is tempting to speculate that the neuroleptic-induced depression of the striato-nigral GABA system may have caused some damage to the neurones, possibly due to disuse degeneration. The present finding of a reduced nigral GAD activity in haloperidol-treated rats and particularly in those displaying a high rate of oral dyskinesias thus seems to link this movement disorder with a hypofunction of GABA-ergic neurones with their terminals in the substantia nigra. Such a lesion may elicit abnormal movements either via nigro-striatal dopamine neurones or through other GABA-modulated nigral efferent systems (cf. Scheel-Krüger et al. 1981; Gale and Casu 1981).

Reduced GAD activity in the substantia nigra has been reported in patients with Parkinson's disease (Rinne et al. 1974; Lloyd et al. 1975; Javoy-Agid et al. 1981). This observation supports the notion that remaining striatal dopamine transmission is essential for the maintenance of intact striato-nigral GABA function. However, in spite of reduced nigral GAD these parkinsonian patients displayed the typical akinesia during their life-time. This favours the view that nigro-striatal dopamine neurones are important for the expression of neuroleptic-induced dyskinetic movements even if the critical lesion lies within the GABA neurone system.

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References

Barany S, Ingvast A, Gunne LM (1979) Development of acute dystonia and tardive dyskinesia in Cebus monkeys. Res Commun Chem Pathol Pharmacol 25:269–279

Bird ED, Iversen LL (1974) Huntington's chorea. Post mortem measurement of glutamic acid decarboxylase, choline acetyltransferase and dopamine in basal ganglia. Brain 97:457–472

Carlsson A (1970) Biochemical implications of dopa-induced actions on the central nervous system with particular relevance to abnormal movements. In: Barbeau A, McDowell FH (eds) L-Dopa and parkinsonism. Davis, Philadelphia, pp 205–213

Gale K (1980) Chronic blockade of dopamine receptors by antischizophrenic drugs enhances GABA binding in substantia nigra. Nature 283:569–570

Gale K, Casu M (1981) Dynamic utilization of GABA in substantia nigra: regulation by dopamine and GABA in the striatum, and its clinical and behavioral implications. Mol Cell Biochem 39:369–405

Glassman RB, Glassman HN (1980) Oral dyskinesia in brain-damaged rats withdrawn from a neuroleptic: implication for models of tardive dyskinesia. Psychopharmacology 69:19–25

Gunne LM, Growdon J, Glaeser B (1982) Oral dyskinesia in rats following brain lesions and neuroleptic drug administration. Psychopharmacology 77:134–139

Häggström JE, Andersson U, Gunne LM (1981) A primate model for tardive dyskinesia. Post mortem biochemical findings. In: Perris C, Struwe G, Jansson B (eds) Developments in psychiatry, vol 5, Biological psychiatry. Elsevier/North Holland, Biomedical Press, Amsterdam, pp 837–839

Itoh M (1983) Effect of haloperidol on glutamine decarboxylase activity in discrete brain areas of the rat. Psychopharmacology 79:169–172

Itoh M, Uchimura H (1981) Regional differences in cofactor saturation of glutamate decarboxylase in discrete brain nuclei of the rat. Neurochem Res 6:1277–1283

Javoy-Agid F, Ploska A, Agid Y (1981) Microtopography of tyrosine hydroxylase, glutamic acid decarboxylase, and choline acetyltransferase in the substantia nigra and ventral tegmental area of control and parkinsonian brains. J Neurochem 37:1218–1227

Kirk RE (1968) Experimental design: Procedures for the behavioral sciences. Brooks/Cole Publishing Company, Belmont, California

Klawans Jr HL (1973) The pharmacology or tardive dyskinesia. Am J Psychiatry 130:82–86

Lloyd KG, Hornykiewicz O (1977) Effect of chronic neuroleptic or L-dopa administration on GABA levels in the rat substantia nigra. Life Sci 21:1489–1496

Lloyd KG, Möhler H, Heitz PH, Bartholini G (1975) Distribution of choline acetyltransferase and glutamate decarboxylase within the substantia nigra and in other brain regions from control and Parkinsonian patients. J Neurochem 25:789–795

McGeer PL, McGeer EG (1976) The GABA system and function of the basal ganglia: Huntington's Disease. In: Roberts E, Chase TN, Tower DB (eds) GABA in nervous system function. Raven Press, New York, pp 487–495

Mao CC, Cheney DL, Marco E, Revuelta A, Costa E (1977) Turnover times of gamma-butyric acid and acetylcholine in nucleus caudatus, nucleus accumbens, globus pallidus and substantia nigra: effects of repeated administration of haloperidol. Brain Res 132:375–379

Mao C, Costa E (1978) Biochemical pharmacology of GABA transmission. In: Lipton MA, DiMascio A, Killam KF (eds) Psychopharmacology: A generation of progress. Raven Press, New York, pp 307–318

Ribak CE, Vaughn JE, Roberts E (1979) The GABA neurons and their axon terminals in rat corpus striatum as demonstrated by GAD immunocytochemistry. J Comp Neurol 187:261–274

Rinne UK, Laaksonen H, Riekkinen P, Sonninen V (1974) Brain glutamic acid decarboxylase activity in Parkinson's disease. Eur Neurol 12:13–19

Scatton B, Worms P, Lloyd KG, Bartholini G (1982) Cortical modulation of striatal function. Brain Res 232:331–343

Scheel-Krüger J, Magelund G, Olianas MC (1981) Role of GABA in the striatal output system: globus pallidus, nucleus entopeduncularis, substantia nigra and nucleus subthalamicus. In: DiChiara G, Gessa GL (eds) GABA and the basal ganglia. Raven Press, New York, pp 165–186

Tarsy D, Baldessarini RJ (1977) The pathophysiologic basis of tardive dyskinesia. Biol Psychiatry 12:431–450

Urquhart N, Perry TL, Hansen S, Kennedy J (1975) GABA content and glutamic acid decarboxylase activity in brain of Huntington's chorea patients and control subjects. J Neurochem 24:1071–1075

Wu JY, Wong E, Saito K, Roberts E, Schousboe A (1976) Properties of L-glutamate decarboxylase from brains of adult and newborn mice. J Neurochem 27:653–659

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