stances is the same. E (caerulein) is the most potent stimulant followed by A (CCK-8), B, C and D. In an additional experiment the analogues were administered in high doses up to 3600 pmoles/kg · h. Under these conditions their effect was similar to the maximal response obtained with CCK-8.

Discussion. CCK-8, like CCK, stimulates mainly pancreatic protein secretion; volume and bicarbonate are stimulated to a much lesser degree. None of the 3 analogues reached the potency of CCK-8 for any parameter of exocrine pancreatic secretion. The substitution of methionine by methoxinine in position 3 (analogue B) already weakens the potency of the octapeptide. This is in contrast to the in vitro results obtained on guinea-pig gall bladder strips in which this analogue showed almost the same EC_{50} as CCK-8[10]. The substitution in position 6 (analogue C) induces a considerable reduction of the biological activity, and consequently with double substitution in positions 3 and 6 most of the activity is lost (analogue D). This reduction was even more pronounced in the in vitro assays[10], where analogues C and D showed only 5 and 2% respectively of the CCK-8 potency. However the sequence of the analogues with respect to potency is the same in both systems. Since all compounds produced the same maximal effect they must be considered full agonists with respect to exocrine pancreatic secretion, differing from each other mainly in their affinity for the receptor. This conclusion is fully supported by the in vitro studies.

The decapeptide caerulein was, on a molar basis, 1.8 times more potent than CCK-8 in stimulating pancreatic protein secretion. This is somewhat lower than the factor 3 published previously by Grossman's group[11].

1 Acknowledgments. The authors are grateful to Dr C. Beglinger for technical cooperation, Prof. G.A. Stalder for his advice and Mrs C. Frei for typing the manuscript. This study was supplied by grant No. 3.816-076 of the Swiss National Foundation for Scientific Research.

2 V. Mutt and J.E. Jorpes, Eur. J. Biochem. *6*, 156 (1968).
3 M.A. Ondetti, B. Rubin, S.L. Engel, J. Pluščec and J.T. Sheehan, Am. J. dig. Dis. *15*, 149 (1970).
4 A. Anastasi, E. Erspamer and R. Endean, Arch. Biochem. *125*, 57 (1968).
5 J.E. Rehfeld, J. biol. Chem. *253*, 4022 (1978).
6 E. Straus and R.S. Yalow, Proc. natl Acad. Sci. USA *75*, 486 (1978).
7 J. Pluščec, J.T. Sheehan, E.F. Sabo, N. Williams, O. Kocy and M.A. Ondetti, J. med. Chem. *13*, 349 (1970).
8 R. de Castiglione, in: Hormonal Receptors in Digestive Tract Physiology, p.33. Ed. S. Bonfils, P. Fromageot and G. Rosselin, North-Holland Publ. Co., Amsterdam 1977.
9 H.M. Greven, A.H.N.M. Bruins and F.A. van der Vlugt, in: Peptides 1971, p.290. Ed. H. Nesvadba. North-Holland Publ. Co., Amsterdam 1973.
10 D. Gillessen, A. Trzeciak, R.K.M. Müller and R.O. Studer, Int. J. Peptide Protein Res. *13*, 130 (1979).
11 H.T. Debas and M.I. Grossman, Digestion *9*, 469 (1973).
12 L. Kayasseh, W.H. Haecki, K. Gyr, G.A. Stalder, W.W. Rittmann, F. Halter and J. Girard, Scand. J. Gastroenterol. *13*, 385 (1978).
13 O. Farooq, R.A.L. Sturdevant and J.I. Isenberg, Gastroenterology *66*, 204 (1974).
14 G.W. Snedecor and W.G. Cochran, Statistical Methods, p.272. The Iowa State Univ. Press, Ames, Iowa, USA 1967.

Supersensitivity after intraventricular 6-hydroxydopamine: Relation to dopamine depletion[1]

M.J. Zigmond and E.M. Stricker

Departments of Biological Sciences and Psychology and the Neuropsychobiology Program, University of Pittsburgh, Pittsburgh (PA 15260, USA), 21 May 1979

Summary. 6-Hydroxydopamine increased behavioral response to L-DOPA in proportion to the decrease of dopamine (DA) and DA uptake in rat striatum. The increased response to apomorphine, however, only occurred after > 80% DA loss. Thus, 6-hydroxydopamine may induce postsynaptic changes only following large lesions.

Animals sustaining large lesions of central dopamine (DA) - containing neurons show increased responsiveness in various behavioral tests to certain dopaminergic agonists, including L-DOPA and apomorphine[2–8]. In rats with different magnitudes of DA depletion, produced by intraventricular administration of the neurotoxin, 6-hydroxydopamine (6-HDA), we have recently found that an increased response to L-DOPA occurred even when the loss of DA was relatively small, whereas an increased response to apomorphine required a large depletion[7,8]. We now explore the basis for these observations and discuss their implications for the recovery of function seen in rats after damage to central dopaminergic neurons.

Male Sprague-Dawley rats (Zivic-Miller Labs) weighing 200–250 g at the start of each experiment were house singly and given food pellets and water ad libitum. 6-HDA hydrobromide (50–250 µg, doses expressed as units of free base) or its vehicle (0.9% NaCl, 0.1% ascorbic acid) was administered into the lateral ventricles[9]. 30 min prior to 6-HDA or vehicle injections, animals were given pargyline (50 mg/kg, i.p.), to potentiate the effects of 6-HDA on dopaminergic terminals, and desmethylimipramine (25 mg/kg, i.p.), to protect noradrenergic terminals from destruction. Most rats appeared normal within 1–2 days. However, some animals receiving the higher doses (200–250 µg) became aphagic and required intragastric intubation of liquid diets and/or access to highly palatable foods for several days before they could maintain their body weight on pelleted chow and water. Behavioral and biochemical measurements were made 40–60 days after lesioning.

When treated with L-DOPA (60 mg/kg, i.p.), most of the 6-HDA-treated animals were more responsive than control rats. In contrast, only animals receiving the largest amounts of 6-HDA were at all responsive to apomorphine (0.05 mg/kg, i.p.). When DA depletions (measured 1 week later[10]) were compared to performance during this behavioral test, the motor activity induced by L-DOPA was found to increase with increasing loss of DA throughout a broad range of brain damage, while an increased response to apomorphine was seen only in animals whose DA depletions were greater than 80% (figure 1). These results confirm and extend our previous studies in which lesion-induced changes in the dose-response curves for these agonists were examined[7,8].

In a 2nd group of rats we assessed high affinity DA uptake[11], an index of DA transport, or DA-stimulated adenylate cyclase[12], an index of target cell responsiveness.

Lesioned animals showed a decreased accumulation of H^3DA (0.5–2.0 μM) into synaptosome-rich P_2 fractions of striatal homogenates. A kinetic analysis indicated that this was attributable to a decrease in the apparent V_{max} which was closely correlated with the change in endogenous DA content of the tissues ($r=0.92$, $p<0.001$). There was no change in the Kt for DA transport (1.3 μM). We also observed a 2-fold increase in the ability of DA to stimulate cAMP production in striatal homogenates prepared from rats with a severe DA depletion of 91±2% (figure 2). On the other hand, in 4 animals with more moderate DA depletions (range, 36–59% depletion) the effect of DA (100 μM) on adenylate cyclase was identical to that observed in control rats.

DA, the presumed active metabolite of exogenous L-DOPA[4], is inactivated primarily by high affinity uptake into dopaminergic terminals[13]. Our observation that in vitro H^3 DA accumulation and responsiveness to L-DOPA are both correlated with the loss of endogenous DA suggests that behavioral "supersensitivity" to L-DOPA resulted from a loss of these uptake sites, especially in animals whose DA depletions did not exceed 80%. In contrast to L-DOPA, apomorphine acts directly on DA receptors[14] and is probably not inactivated by high affinity transport. Thus, increased responsiveness to apomorphine is presumably indicative of an increase in the sensitivity of target cells to dopaminergic agonists. Our behavioral and biochemical findings suggest that this occurs only after extensive loss of DA, and comparably large DA depletions also were present in all previous studies indicating postsynaptic supersensitivity[2,3,5,15,17].

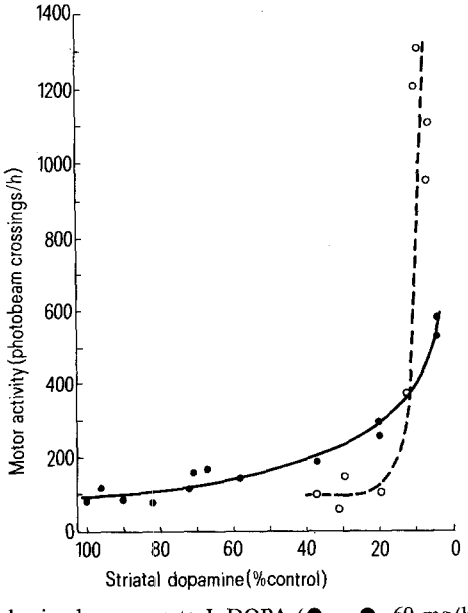

Fig. 1. Behavioral response to L-DOPA (●——●, 60 mg/kg, i.p.) and apomorphine (○ – – – ○, 0.05 mg/kg, i.p.) as a function of residual DA in rats lesioned with 6-HDA (50–250 μg, intraventricular) 30 min after desmethylimipramine (25 mg/kg, i.p.) and pargyline (50 mg/kg, i.p.). Motor activity was measured in individual cages (38 $cm^2 \times 17.5$ cm high) equipped with a photobeam 6.5 cm above the floor. Photobeam interruptions were counted for 1 h beginning 5 min after drug administration. All animals given L-DOPA had received RO4-4602 (50 mg/kg, i.p.) 30 min previously to inhibit peripheral decarboxylase. Basal activity was unaffected by vehicle injections (0.9% NaCl, with 1% asorbic acid for L-DOPA) and was similar for control and lesioned rats. Dopamine levels are presented as a percentage of the mean control value (9.4 μg/g fresh weight). The depletion of norepinephrine in whole telencephalon did not exceed 13% in any animal. Each value represents an individual animal.

The behavioral effects of 6-HDA-induced lesions depend largely on the extent of the DA loss. Rats having DA depletions smaller than 90–95% have few gross dysfunctions, whereas rats with larger lesions become akinetic and show marked sensory neglect. These symptoms may gradually diminish, but residual behavioral deficits to acute, intense challenges persist. For example, such animals no longer increase their food intake in response to insulin or 2-deoxy-D-glucose, nor do they drink during the cellular dehydration which follows the administration of hypertonic saline (see Stricker and Zigmond[18] for review). Our present findings have several implications for these observations. First, the absence of an increase in target cell sensitivity in animals with DA depletions less than 80% suggests that the postsynaptic cells continue to receive dopaminergic input. This may be facilitated by the increase in DA turnover in residual terminals[19,20], and by an increase in diffusion of DA resulting from the loss of reuptake sites. Second, the apparent increased responsiveness of these cells in animals with DA depletions exceeding 80% suggests an early period of reduced DA receptor activation. The resulting disruption in dopaminergic transmission may underlie the gross behavioral dysfunctions initially observed after the lesion, while the progressive development of increased postsynaptic sensitivity[2,8] may contribute to the gradual recovery of function. Finally, the inability of lesioned animals to behave appropriately when stressed may indicate that the presence of postsynaptic supersensitivity does not insure the return of normal function. We have observed that exposure to

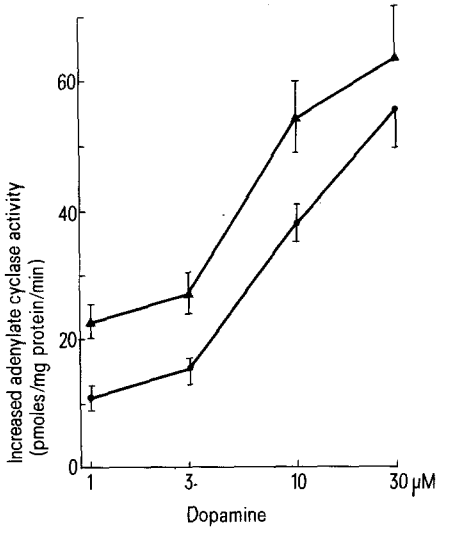

Fig. 2. DA-sensitive adenylate cyclase activity in control animals (●——●) and animals treated with 150–250 μg 6-HDA (▲——▲) (average DA depletion, 91% of control). The striatum was homogenized in 80 mM Tris maleate, pH 7.4 containing 2 mM $MgSO_4$ and 0.5 mM EGTA and was incubated at 30°C with aminophylline (10 mM), phosphocreatinine (20 mM), creatinine phosphokinase (100 units/ml) and ATP-^{14}C (1 mM; 0.5 μCi), each component being made up in the Tris maleate $MgSO_4$-EGTA buffer (final volume, 120 μl). After 2.5 min, the reaction was terminated by boiling for 3 min. (Blanks were boiled without prior incubation). Next, 800 μl H_2O was added to each tube and the sample transferred to an alumina column (1 g unpurified alumina equilibrated with 60 mM Tris-HCl buffer, pH 7.4 and placed on a 8 mm inner diameter column). The sample was then eluted with 2 ml Tris-HCl onto a Dowex column (3 cm × 8 mm of Dowex AG 50W resin previously washed with 1 M NaOH and 1 M HCl and then with H_2O until neutral). The Dowex was washed with approximately 1.8 ml H_2O and then eluted directly into counting vials with approximately 4 ml H_2O. Values are the increase in adenylate cyclase activity above baseline, which was 104.1±3.3 pmoles/mg protein/min for control rats, 104,0±5.6, for 6-HDA rats. Each value represents a mean ± SEM for 12 animals.

stressors such as 2-deoxy-D-glucose is accompanied by an increase in DA turnover[21,22] while the normal behavioral response to such challenges is blocked by DA receptor antagonists[23,24]. Perhaps the persistent behavioral deficits seen in lesioned rats result because of an inability to provide sufficient DA in response to increased need.

1 Supported in part by USPHS grants No. MH-00058 and MH-20620. We thank Kathleen Hays, Suzanne Wuerthele and Dennis McKeag for technical assistance.
2 U. Ungerstedt, Acta physiol. scand., suppl. *367,* 69 (1971).
3 R.I. Schoenfeld and N.J. Uretsky, Eur. J. Pharmac. *19,* 115 (1972).
4 R.I. Schoenfeld and N.J. Uretsky, J. Pharmac. exp. Ther. *186,* 616 (1973).
5 P.F. Von Voigtlander and K.E. Moore, Neuropharmacology *12,* 451 (1973).
6 J.E. Thornburgh and K.E. Moore, J. Pharmac. exp. Ther. *192,* 42 (1975).
7 M.J. Zigmond and E.M. Stricker, in: Chemical Tools in Catecholamine Research, vol. 1, p. 319. Ed. G. Jonsson, T. Malmfors and Ch. Sachs. North-Holland, Amsterdam 1975.
8 M.J. Zigmond and E.M. Stricker, in: Animals Models in Psychiatry and Neurology, p. 415. Ed. I. Hanin and E. Usdin. Pergamon, New York 1977.
9 M.J. Zigmond and E.M. Stricker, Science *177,* 1211 (1972).
10 C.F. Saller and M.J. Zigmond, Life Sci. *23,* 1117 (1978).
11 M.J. Zigmond, J.P. Chalmers, J.P. Simpson and R.J. Wurtman, J. Pharmac. exp. Ther. *179,* 20 (1971).
12 A. Guidotti and E. Costa, J. Pharmac. exp. Ther. *189,* 665 (1974).
13 J.T. Coyle and S.H. Snyder, J. Pharmac. exp. Ther. *170,* 221 (1969).
14 A.A. Ernst, Psychopharmacology *10,* 316 (1967).
15 R.K. Mishra, E.L. Gardner, R. Katzman and M.H. Makman, Proc. nat. Acad. Sci. *71,* 3883 (1974).
16 H.C. Fibiger and D.S. Grewaal, Life Sci. *15,* 57 (1974).
17 I. Creese, D.R. Burt and S.H. Snyder, Sci. *197,* 596 (1977).
18 E.M. Stricker and M.J. Zigmond, in: Progress in Psychobiology and Physiological Psychology, vol. 6, p. 121. Ed. J. Sprague and A.N. Epstein. Academic Press, New York 1976.
19 Y. Agid, F. Javoy and J. Glowinski, Nature New Biol. *243,* 150 (1973).
20 A.L. Acheson, M.J. Zigmond and E.M. Stricker, Trans. Am. Soc. Neurochem. *10,* 142 (1979).
21 L. Kennedy, C. Saller and M. Zigmond, Neurosci. abstr. *5,* 339 (1979).
22 A.M. Thierry, J.P. Tassin, G. Blanc and J. Glowinski, Nature *263,* 242 (1976).
23 M.L. Block and A.E. Fisher, Pharmac. Biochem. Behav. *3,* 251 (1975).
24 A.P. Zis and H.C. Fibiger, Psychopharmacologia *43,* 63 (1975).

Effect of fluoride administration on renal glucose-6-phosphatase activity in rats[1]

Y. Suketa, M. Sato and M. Kura

Department of Environmental Biochemistry, Shizuoka College of Pharmacy, Oshika, Shizuoka (Japan), 28 May 1979

Summary. Renal glucose-6-phosphatase activity was found to be significantly elevated by fluoride administration (NaF 35 mg/kg, i.p.). The elevation of the enzyme activity was markedly suppressed by adrenalectomy.

Taylor et al[2]. reported an increase in urinary excretion of glucose after a dose of fluoride (NaF 20-30 mg/kg, i.v.). Shearer[3] found increases in kidney glucose in rats receiving 25 mg/kg NaF i.p. Net glucose liberation from gluconeogenic tissues of the liver and kidney was believed to be controlled through glucose-6-phosphatase[4]. The present study was designed to examine the effect of fluoride on the renal glucose-6-phosphatase activity in rats given a single dose of NaF (i.p.).

Materials and methods. Male Wistar albino rats weighing about 100 g were maintained on the MF basal diet (purchased from Oriental Yeast Ind., Japan) and water ad libitum. The animals were maintained at a temperature of 22 °C for a minimum of a week. All animals were fasted for 24 h before the experiments in order to minimize the effects of glucose absorption from the bowels and to stabilize the urinary excretion of glucose. The rats were sacrificed at various intervals after a single i.p. administration of NaF (0, 10, 20 or 35 mg/kg). NaF (35 mg/kg, i.p.) was injected into rats 2 days after adrenalectomy or parathyroidectomy. Microsomes were prepared according to the method of Jørgensen[5]. At the time of sacrifice, the rats were anesthetized with ether and killed by cardiac puncture. The kidneys were removed and the tissues (1 g) were immediately homogenized in a Potter-Elvehjem teflon-glass homogenizer with 5 ml of ice-cold 0.25 M sucrose-0.03 M histidine buffer (pH 7.2). Reproducible preparations of the heavy microsomal fraction were obtained by centrifugation (25,300 × g, 30 min) of supernatant after sedimentation of the mitochondria at 10,800 × g for 30 min. The preparations of the heavy microsomal fraction (1 mg of protein/ml of 0.25 M sucrose-0.03 M histidine buffer, pH 7.2) were stored in a refrigerator (−20 °C). Glucose-6-phosphatase activity was determined according to the method of Swanson[6]. Protein was determined by the method of Lowry et al.[7]. Glucose-6-phosphate (disodium salt) was obtained from the Sigma Chemical Co. (St. Louis, USA).

Results and discussion. The responses of renal glucose-6-phosphatase activity to different doses of fluoride were examined as shown in the figure, a). Increasing doses of fluoride caused a more pronounced increase in renal glucose-6-phosphatase activity. The changes in renal glucose-

Effect of fluoride administration on renal glucose-6-phosphatase activity in intact, adrenalectomized and parathyroidectomized rats

Treatment		Glucose-6-phosphatase activity (moles/mg protein/min)	T/N
Intact	None (normal)	102.1± 20.2	1
	Fluoride	300.3± 131.5	2.94*
PTX	None	36.4± 9.4	0.36* (1)
	Fluoride	105.7± 25.7	1.04 (2.90)
AX	None	60.7± 6.7	0.59* (1)
	Fluoride	46.0± 1.2	0.45* (0.76)

The animals were killed 3 h after the fluoride dose (35 mg/kg, i.p.). Values are averages obtained from 4-6 rats ± SE. T/N: ratio of treatment of fluoride or - ectomy against normal rats, *P < 0.01. Numbers in parentheses are relative values of respective controls. PTX: parathyroidectomized rats, AX: adrenalectomized rats.