ing times). So far, our results with aprophen are in congruence with those obtained with other compounds[3-5,23], and show that aprophen acts diphasically on hepatic microsomal drug metabolism. But in contrast to those compounds which do not inhibit cytochrome c reductase, the activity of this enzyme is decreased 30 min after aprophen administration. In this respect, 2,2-diphenylpropionic acid N,N-diethylaminoethyl ester hydrochloride behaves like N,N-diethylacetamide in that both demethylases and cytochrome c reductase are decreased shortly after the administration of the compound[24]. In the case of both substances, there is no inhibition of cytochrome c reductase in vitro[8,24]. This means that with aprophen and diethylacetamide, drug metabolism inhibition as found in in vivo experiments is not only confined to an interaction of the drug metabolism inhibitors with cytochrome P-450 as it is observed with 2-hydroxy-2-ethylbutyryl N,N-diethylamide[25] and is discussed for the other drugs mentioned earlier[7], but that there are additional effects of these 2 compounds on microsomal drug-metabolizing enzymes which lead to a decrease of the cytochrome reductase, too, but these additional effects do not occur in vitro. In vitro, aprophen is shown to inhibit mixed-function oxidases but not cytochrome c reductase[8]. This to lesser extent, is the case with the free 2,2-diphenylpropionic acid[26]. N,N-diethylacetamide has no effect at all on microsomal drug metabolism in vitro[24]. The nature and the toxicological consequences of these different effects in vivo and in vitro are being studied.

There is another striking result which only can be pointed to but cannot be discussed at length, namely the lack of inverse proportionality between hexobarbital sleeping time and drug-metabolizing enzyme activity. At first glance, one might expect such an inverse proportionality to occur theoretically. The same lack of inverse proportionality was observed with 2-hydroxy-2-ethylbutyryl N,N-diethylamide[3] and with the action times of a variety of other drugs, too (unpublished results). The proper explanation for these effects have yet to be found although some first approximating steps already exist[27].

1 Jaffe, H., Fuji, K., Guerin, H., Sengupta, M., and Epstein, S., Biochem. Pharmac. *18* (1969) 1045.
2 Kramer, M., and Arrigoni-Martelli, E., Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak. *237* (1959) 264.
3 Beyhl, F.E., and Lindner, E., Experientia *32* (1976) 362.
4 Lindner, E., and Beyhl, F.E., Experientia *34* (1978) 226.
5 Beyhl, F.E., and Lindner, E., 6th Eur. Workshop Drug Metabolism, Leiden (Netherlands), June 1973, abstr.
6 Goldstein, D.B., and Goldstein, A., Biochem. Pharmac. *8* (1961) 48.
7 Beyhl, F.E., in: Zur Problematik von chronischen Toxizitätsuntersuchungen, p.52. Eds B. Schnieders and D. Grosdanoff. Dietrich Reimer Verlag, Berlin 1980.
8 Beyhl, F.E., and Sinharay, A., Naunyn-Schmiedeberg's Arch. Pharmak. Suppl. *319* (1982) R11.
9 Axelrod, J., Reichenthal, J., and Brodie, B.B., J. Pharmac. exp. Ther. *112* (1954) 49.
10 Cooper, J.R., Axelrod, J., and Brodie, B.B., J. Pharmac. exp. Ther. *112* (1954) 55.
11 Jenner, S., and Netter, K.J., Biochem. Pharmac. *21* (1972) 1921.
12 Zaugg, H.E., and Horrom, B.W., J. Am. chem. Soc. *72* (1950) 3004.
13 Lindner, E., Habilitation thesis. University of Giessen, Giessen 1960.
14 Potter, V.E., and Elvehjem, C.A., J. biol. Chem. *114* (1936) 495.
15 Schenkman, J.B., and Cinti, D.L., Life Sci. *11* (1972) 247.
16 Leber, H.W., Degkwitz, E., and Staudinger, H., Hoppe-Seyler's Z. physiol. Chem. *350* (1969) 439.
17 Beyhl, F.E., and Sinharay, A., in: Microsomes, drug oxidations, and chemical carcinogenesis, p.111. Eds M.J. Coon, A.H. Conney, R.W. Estabrook, H.V. Gelboin, J.R. Gillette and P.J. O'Brien. Academic Press, New York 1980.
18 Beyhl, F.E., in: Developments in biochemistry, vol.13, p.141. Eds J.-A. Gustafsson, J. Carlstedt-Duke, A. Mode and J. Rafter. Elsevier/North Holland, Amsterdam 1980.
19 Cleveland, P.D., and Smuckler, E.A., Proc. Soc. exp. Biol. Med. *180* (1965) 808.
20 Nash, T., Biochem. J. *55* (1953) 416.
21 Cochin, J., and Axelrod, J., J. Pharmac. exp. Ther. *125* (1959) 105.
22 Ther, L., Grundlagen der experimentellen Arzneimittelforschung, p.39. Wissenschaftl. Verlagsges., Stuttgart 1965.
23 Kato, R., Vassanelli, P., and Chiesara, E., Experientia *18* (1962) 453.
24 Beyhl, F.E., and Lindner, E., Food Cosm. Toxic. *19* (1981) 627.
25 Beyhl, F.E., Réunion Commune de Biochimie, Strasbourg (France), Sept. 1981., abstr. p.75.
26 Beyhl, F.E., IRCS med. Sci. *10* (1982) 218.
27 Beyhl, F.E., Naunyn-Schmiedeberg's Arch. Pharmac., Suppl. *307* (1979) R9.

Inhibition of neurotensin (NT)-induced glucagon release by [D-Trp^{11}]-NT[1]

M. Ukai, T. Itatsu, A. Shibata, F. Rioux and S. St-Pierre

Institute of Germfree Life Research, Nagoya University School of Medicine, Showa-ku, Nagoya 466 (Japan), and Department of Physiology and Pharmacology, Faculty of Medicine, University of Sherbrooke, Sherbrooke (Quebec, Canada),

19 February 1982

Summary. A synthetic analog of neurotensin (NT), [D-Trp^{11}]-NT, antagonized NT-induced hyperglucagonemia and hyperglycemia, and also NT-induced glucagon release from pancreatic islets in rats.

The tridecapeptide neurotensin (NT)[2,3] with the structure of pGlu^1-Leu^2-Tyr^3-Glu^4-Asn^5-Lys^6-Pro^7-Arg^8-Arg^9-Pro^{10}-Tyr^{11}-Ile^{12}-Leu^{13}-OH has been found to be distributed in the brain, gut, pancreas and blood[4-6], and to exhibit a variety of biological effects, including hyperglucagonemia[7-9] and others[10].

Recent structure-activity studies using NT and its analogs have yielded evidence to support the view that the C-terminal part (hexapeptide) of the molecule is essential for the biological activity of NT[11-17] and that the chemical structure of Tyr^{11} may play a critical role in the process of NT receptor activation[13,16,17]. It is of special interest that [D-Trp^{11}]-NT antagonized selectively NT-induced coronary vasoconstriction in rats, but displayed NT-like activity in higher concentrations[17].

The present work was undertaken to investigate the effect

Table 1. The interaction of neurotensin (NT) and [D-Trp[11]]-NT on plasma glucose, glucagon (IRG) and insulin (IRI) levels in rats

Group	Peptide injected	No. of rats	Glucose (mg/dl)	IRG (pg/ml)	IRI (μU/ml)
A	none	8	101.9±3.5	106.1± 9.4	18.4±3.9
B	NT 10^{-6} M	6	125.2±3.5[a]	152.9±18.7[a]	12.9±1.9
C	NT 10^{-6} M + [D-Trp[11]]-NT 10^{-5} M	7	107.4±3.8[b]	91.1± 9.1[b]	11.6±2.1
D	[D-Trp[11]]-NT 10^{-5} M	7	102.3±4.8	96.7±11.5	19.9±0.9

Values are expressed as the mean ± SE. Blood samples were collected by decapitation 5 min after i.v. injection of saline or the peptide solution (1.5 ml/kg b.wt) in the group A, B or D. In the group C, the analog was given 5 min prior to NT administration. Significant differences ($p < 0.05$) from the group A and B values are indicated by a and b, respectively.

Table 2. The interaction of neurotensin (NT) and [D-Trp[11]]-NT on glucagon (IRG) and insulin (IRI) release from rat pancreatic islets

Group	Peptide in medium	No. of experiments	IRG (pg/islet/30min)	IRI (μU/islet/30min)
A	none	10	88.5±4.4	18.6±0.6
B	NT 10^{-6} M	10	136.3±9.4[a]	17.5±0.9
C	NT 10^{-6} M + [D-Trp[11]]-NT 10^{-5} M	10	103.2±5.3[ab]	19.7±1.1
D	[D-Trp[11]]-NT 10^{-5} M	8	106.1±9.7	16.3±0.8

Values are expressed as the mean ± SE. Significant differences ($p < 0.05$) from the group A and B values are indicated by a and b, respectively.

of [D-Trp[11]]-NT on NT-induced glucagon release in vivo and in vitro in rats.

Materials and methods. Adult Sprague-Dawley rats weighing 150–250 g were used. In experiments in vivo, NT (10^{-6}M) or [D-Trp[11]]-NT (10^{-5}M) dissolved in saline was administered in a volume of 1.5 ml/kg b.wt through the trail vein under pentobarbital anesthesia (45 mg/kg b.wt) to male rats which had been fasted overnight. The analog was given 5 min prior to NT administration. Blood was collected by decapitation 5 min after administration of NT or saline (as control), into heparinized tubes containing a mixture (0.1 ml) of Trasylol (1000 U, Bayer Ltd) and di-sodium ethylenediamine tetraacetic acid (EDTA, 1.2 mg) per ml blood. Plasma was separated immediately and stored at −20 °C until assays. In experiments in vitro, pancreatic islets were isolated from fed rats under pentobarbital anesthesia, using the collagenase technique, and incubated at 37 °C as described previously[18–20]. That is, batches of 10 islets were preincubated for 60 min in 3 ml of a Krebs-Ringer bicarbonate medium (pH 7.4) containing 16.7 mM glucose, and then incubated for 30 min in 3 ml of the medium containing 5.5 mM glucose with and without test substances in concentrations indicated in the results. At the end of 30-min incubation, 2 samples of 0.4 ml from each medium were collected in chilled tubes containing 0.2 ml of the Trasylol-EDTA mixture above described and kept frozen as in the plasma. Immunoreactive glucagon (IRG) and insulin (IRI) concentrations in the plasma or medium were determined using 30K antibody and the solid phase method, respectively[8,19,20]. Porcine glucagon (Eli Lilly and Co.) was used as the standard. NT (Protein Research Foundation, Osaka), [D-Trp[11]]-NT (University of Sherbrooke)[17] and L-arginine-HCl (Nagoya Katayama Chemical Co.) were employed as test substances.

Results and discussion. Intravenous (i.v.) administration of NT (1.5 nmoles/kg b.wt) to rats caused a significant increase in plasma glucagon and glucose at 5 min (table 1), consistent with previous results of studies in rats[7,9] and dogs[8] while it tended to lower plasma insulin levels. Pretreatment with [D-Trp[11]]-NT in a 10-fold higher dose 5 min prior to NT, completely blocked the NT-effect on plasma glucagon and significantly inhibited that on plasma glucose, while it did not affect plasma insulin levels after NT. [D-Trp[11]]-NT alone in a dose of 15 nmoles/kg b.wt had no effects on plasma glucagon, glucose and insulin levels, as understood by comparison of the group A and D values (table 1). The results on plasma glucose and glucagon are consistent with the view that NT-induced hyperglycemia is mainly glucagon-dependent[8] and partially ascribed to other factors including catecholamines[21], histamin[9,20,22] and insulin[7]. In addition, [D-Trp[11]]-NT appears to act on the process of glucagon release, and not on insulin release, as a selective antagonist of NT in the present experimental conditions.

In experiments with pancreatic islets, L-arginine (10 mM) significantly stimulated glucagon release (pg/islet/30 min) from the control 88.5±4.4 (mean±SE) to 205.7±17.1 (N=10) and insulin release (μU/islet/30 min) from the control 18.6±0.6 to 33.1±1.5 (N=10).

In this in vitro system, NT (10^{-6}M) in the medium stimulated glucagon release and exerted no significant effect on insulin release (table 2), being consistent with our previous studies[19,20,22]. However, [D-Trp[11]]-NT (10^{-5}M) significantly inhibited the NT effect on glucagon release, as seen in the group C value. [D-Trp[11]]-NT (10^{-5}M) alone did not significantly affect glucagon release from the islets, being suggestive of its agonistic nature[17] on glucagon release. On the other hand, insulin release was not affected significantly by these peptides (table 2). The data on experiments in vitro may be interpreted as indicating that the interaction of NT and [D-Trp[11]]-NT may occur in the endocrine pancreas, by the mechanism of probable competition of both peptides in the process of binding to and activation of the receptors involved in glucagon release from the A cells.

1 This work was supported in part by a grant from the Imanaga Medical Research Foundation, Nagoya, Japan. Address for reprint requests: M.U., Department of Surgery, National Nagoya Railway Hospital, Taiko 1-chome, Nakamura-ku, Nagoya 453, Japan.

2 Carraway, R., and Leeman, S.E., J. biol. Chem. *248* (1973) 6854.

3 Carraway, R., and Leeman, S.E., J. biol. Chem. *250* (1975) 1912.

4 Carraway, R., and Leeman, S.E., J. biol. Chem. *251* (1976) 7045.

5 Polak, J.M., Sullivan, S.N., Bloom, S.R., Buchan, A.M.J., Facer, P., Brown, M.R., and Pearce, A.G.E., Nature *270* (1978) 183.

6 Blackburn, A.M., Bryant, M.G., Adrian, T.E., and Bloom, S.R., J. clin. Endocr. Metab. *52* (1981) 820.

7 Brown, M., and Vale, W., Endocrinology *98* (1976) 819.
8 Ukai, M., Inuoue, I., and Itatsu, T., Endocrinology *100* (1977) 1284.
9 Nagai, K., and Frohman, L.A., Diabetes *27* (1978) 577.
10 Sasaki, H., Medicina *16* (1979) 2228.
11 Segawa, T., Hosokawa, M., Kitagawa, K., and Yajima, H., J. Pharm. Pharmac. *29* (1977) 57.
12 Lazarus, L.H., Perrin, M.H., Brown, M.R., and Rivier, J.E., Biochem. biophys. Res. Commun. *76* (1977) 1079.
13 Rivier, J.E., Lazarus, L.H., Perrin, M.H., and Brown, M.R., J. med. Chem. *20* (1977) 1409.
14 Loosen, P.T., Nemeroff, C.B., Burnett, G.B., Prange, Jr, A.J., and Lipton, M.A., Neuropharmacology *17* (1978) 109.
15 Quirion, R., Regoli, D., Rioux, F., and St-Pierre, S., Br. J. Pharmac. *68* (1980) 83.
16 Quirion, R., Regoli, D., Rioux, F., and St-Pierre, S., Br. J. Pharmac. *69* (1980) 689.
17 Quirion, R., Rioux, F., Regoli, D., and St-Pierre, S., Eur. J. Pharmac. *61* (1980) 309.
18 Lacy, P.E., and Kostianovsky, H., Diabetes *16* (1967) 35.
19 Shibata, A., Itatsu, T., and Ukai, M., Igaku No Ayumi *114* (1980) 225.
20 Itatsu, T., Shibata, A., and Ukai, M., Endocr. jap. *28* (1981) 31.
21 Nagai, K., and Frohman, L.A., Life Sci. *19* (1976) 273.
22 Ukai, M., Itatsu, T., and Shibata, A., 6th Int. Congr. Endocrinology, Melbourne 1980; abstract 508, p. 463.

Multiple daily amphetamine administration decreases both [^{3}H]agonist and [^{3}H]antagonist dopamine receptor binding[1]

D.R. Sibley, S. Weinberger, D.S. Segal and I. Creese[2]

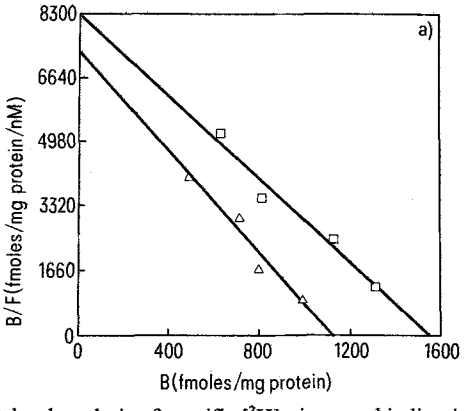
Department of Neurosciences and Department of Psychiatry, University of California, San Diego, La Jolla (California 92093, USA), 22 March 1982

Summary. Multiple daily amphetamine injections in rats decreased both [^{3}H]agonist as well as [^{3}H]antagonist striatal dopamine receptor binding. Concurrently, these animals exhibited a decrease in striatal dopamine concentration and, paradoxically, an enhancement of behavioral responsivity.

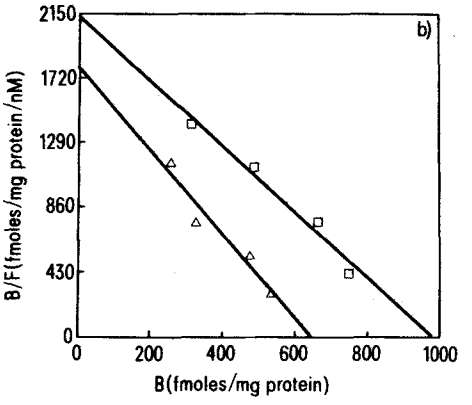
It has previously been shown that multiple injections of d-amphetamine in rats results in an enhanced responsivity to this drug[3-6]. Similar observations have been made in other species[7,8] and in man[9]. Chronic treatment of rats with dopamine receptor antagonists also leads to an augmentation in the behavioral response to dopamine agonists[10] which is accompanied by an increase in striatal dopamine receptor binding of both [^{3}H]agonists and [^{3}H]antagonists[11]. The paradoxical observation of increased behavioral sensitivity to agonists following either chronic agonist or antagonist treatment led us to investigate the effect of multiple daily doses of amphetamine on the dopamine receptor binding of both [^{3}H]agonist and [^{3}H]antagonist ligands.

Material and methods. Male Wistar rats (325-375 g) were treated with 30 successive, s.c. injections of either saline or d-amphetamine sulfate (2.5 mg/kg) at 4-h intervals for 5 days. The animals were sacrificed 4 h after the last injection by decapitation. The brains were quickly removed into ice-cold saline, the striata dissected out and frozen at −70 °C. The binding of the radiolabeled antagonist [^{3}H]spiperone (25.6 Ci/mmole, NEN) and the radiolabeled agonist [^{3}H]N-n-propylnorapomorphine ([^{3}H]NPA, 75 Ci/mmole, NEN) were assayed as previously described in detail[12-14]). Nonspecific binding was determined with 10^{-6}M (+)butaclamol and represented 10-20% of the total [^{3}H]spiperone binding and 30-40% of the total [^{3}H]NPA binding. All experiments were performed in duplicate. Protein was determined by the method of Bradford[15].

Results and discussion. The specific dopamine receptor binding of both [^{3}H]spiperone and [^{3}H]NPA was reduced following the chronic amphetamine treatment (figs a and b; table). The maximum binding capacity (B_{max}) for [^{3}H]spiperone was reduced by about 20% while the B_{max} of the agonist ligand, [^{3}H]NPA, was reduced by about 27%. Although the affinity of [^{3}H]spiperone for its binding sites was unaltered by the treatment the dissociation constant (K_d) for [^{3}H]NPA was slightly reduced from 0.51 to 0.40 nM ($p < 0.05$). Behavioral observations were made on a similar



a Scatchard analysis of specific [^{3}H]spiperone binding in striata of amphetamine (△) and saline (□) treated rats. The left and right striata from each individual animal were pooled and assayed for [^{3}H]spiperone and [^{3}H]NPA binding as described. The results for 2 individual animals assayed in parallel are shown. [^{3}H]spiperone concentrations were varied between 100 pM and 1 nM. The lines drawn represent the best fit to the data as determined by linear regression analysis.



b Scatchard analysis of specific [^{3}H]NPA binding in striata of amphetamine (△) and saline (□) treated rats. The saturation experiments were performed as in figure (a) varying the [^{3}H]NPA concentrations between 200 pM and 2 nM. Data from 2 individual animals assayed in parallel are shown.