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Cloning of the cocaine-sensitive bovine dopamine transporter

(neurotransmitter uptake/substantia nigra/central nervous system)

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ABSTRACT **A cDNA encoding the dopamine transporter from bovine brain substantia nigra was identified on the basis of its structural homology to other, recently cloned, neurotransmitter transporters. The sequence of the 693-amino acid protein is quite similar to those of the rat γ-aminobutyric acid, human norepinephrine, and rat serotonin transporters. Dopamine transporter mRNA was detected by *in situ* hybridization in the substantia nigra but not in the locus coeruleus, raphe, caudate, or other brain areas. [^{3}H]Dopamine accumulation in tissue culture cells transfected with the cDNA was inhibited by amphetamine, cocaine, and specific inhibitors of dopamine transport, including GBR12909.**

Brain dopamine systems play a central role in the control of movement, hormone release, and many complex behaviors (1–4). Drugs that affect dopamine neurotransmission are used to treat psychotic syndromes including schizophrenia, as well as Parkinson disease and other movement disorders. Reuptake into presynaptic terminals is thought to be the major means of terminating the synaptic action of monoamine neurotransmitters (5–7), including dopamine (8). The dopamine reuptake site, or transporter, has been characterized by measuring the uptake of [^{3}H]dopamine and the binding of radioligands (8–13). In addition to its normal physiological function, it appears to be the site most related to the behavioral effects of amphetamine and cocaine (8, 14, 15).

The transporters for γ-aminobutyric acid (GABA) (16, 17), norepinephrine (18) and serotonin (36) have recently been cloned. Their primary sequences and predicted structures are very similar and are quite distinct from both neurotransmitter receptors and other transporters. They thus comprise a new neurotransmitter transporter family. We have now identified and characterized a cDNA clone for the bovine dopamine transporter, which is also a member of this family.‡

MATERIALS AND METHODS

cDNA Library Construction and Screening. A cDNA library of 4×10^6 recombinants was synthesized (19) from bovine substantia nigra poly(A)$^+$ RNA in the vector pCDSP6T7 [derived from pCD (19) by addition of recognition sites for bacteriophage SP6 and T7 RNA polymerases 5′ and 3′ to the cDNA insert; M.J.B., unpublished work]. DNA prepared from 36 subdivisions of 5×10^5 clones from the amplified library was screened on Southern blots (20, 21). The oligodeoxynucleotide probe [5′-TAGGGGATCAGGAAGGCGCCICC(G/A)TT(C/T)TTI(T/C)(A/C)(G/A)CACAGGTAGGGGAACCGCCACACATT-3′] (36) was 3′ end-labeled with ^{32}P using polynucleotide kinase and hybridized to the blots in 6× SSC (1× SSC is 0.15 M NaCl/0.015 M sodium citrate, pH 7.4)/2× Denhardt's solution (1× Denhardt's solution is 0.02% polyvinylpyrrolidone/0.02% Ficoll/0.02% bovine serum albumin) overnight at 60°C. Final wash conditions were 6× SSC at 60°C. Successively smaller subdivisions were screened until a single clone was obtained. Both strands of the cDNA clone were sequenced from single-stranded DNA by the dideoxynucleotide method, using a combination of fragments subcloned into M13 bacteriophage and pBluescript (Stratagene), with a Sequenase kit (United States Biochemical). Fragments were assembled and the sequence was analyzed using standard computer software (22).

RNA Blot Analysis. Antisense oligodeoxynucleotide probes for the dopamine transporter (5′-GAGAAGGCGATGAGCACCCCAAGGCCCACGCCCAGCGAGAAGC-3′) and bovine tyrosine hydroxylase (5′-CATGGGCCAGGGCCTGCATCTCGTCCTGGACGCCGTCCAGGGCGCGCC-3′; ref. 23) sequences were 3′ end-labeled with [α-[^{35}S]thio]dATP and hybridized to cryostat-cut sections of bovine brain as described (24). For Northern blots, mRNA (19) from bovine tissues was enriched by a single passage over oligo(dT)-cellulose, separated according to size in a 1.2% agarose/formaldehyde gel, and transferred by electrophoresis to Nytran membrane (Schleicher & Schuell). The oligonucleotide probe was labeled with [α-^{32}P]dATP using terminal deoxynucleotidyltransferase and incubated with the blot overnight at 37°C in 4× SSPE (1× SSPE is 0.15 M NaCl/0.01 M NaH_2PO_4/1.3 mM EDTA, pH 7.4)/5× Denhardt's solution/50% formamide/0.1% SDS containing denatured salmon sperm DNA (500 μg/ml) and yeast tRNA (250 μg/ml). Final wash conditions were 1× SSPE/0.1% SDS at 60°C.

Uptake Studies. CV-1 cells (monkey kidney cell line) plated in 35-mm (4.5×10^5 cells) or 25-mm (1.4×10^5 cells) tissue culture plates were infected the day after plating with a recombinant vaccinia virus encoding bacteriophage SP6 RNA polymerase (T.B.U., M.J.B., B. Moss, and S. Isaacs, unpublished work) at a multiplicity of infection of 5 plaque-forming units per cell. After 30 min the cells were transfected with dopamine transporter plasmid (0.5 μg/ml) by using TransfectACE (10 μg/ml; BRL) as described for coinfection/transfection using the bacteriophage T7 recombinant vaccinia virus (25, 26). After 24 hr the cells were washed with uptake buffer (25 mM Hepes, pH 7.4/125 mM NaCl/4.8 mM KCl/1.2 mM KH_2PO_4/1.3 mM $CaCl_2$/1.2 mM $MgSO_4$/5.6 mM glucose/1 mM sodium ascorbate/10 μM pargyline), preincubated in uptake buffer for 10 min, and then incubated with [^{3}H]dopamine (New England Nuclear; 32 Ci/mmol; 1 Ci = 37 GBq) for 9 min at 37°C followed by three washes on ice. Various concentrations of [^{3}H]dopamine were used for dose–response curves. K_i values were determined using 100 nM [^{3}H]dopamine, which typically gave 31,000–46,000 dpm total uptake, 1400–3600 dpm nonspecific uptake (defined in the presence of 1 μM GBR12909), and 1200–3300 dpm in mock-

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Abbreviation: GABA, γ-aminobutyric acid.

‡The sequence reported in this paper has been deposited in the GenBank data base (accession no. M80234).

transfected cells. Cells were solubilized in 0.5 M NaOH and radioactivity was determined by liquid scintillation counting.

RESULTS AND DISCUSSION

A cDNA clone for the bovine dopamine transporter was identified and purified by screening a bovine substantia nigra cDNA library at low stringency with a degenerate oligonucleotide probe that was based on sequences shared by the norepinephrine (18) and GABA (16) transporters and that hybridizes to the serotonin transporter cDNA (36). The cDNA clone contained an insert of 2340 nucleotides (sequence deposited in GenBank‡). The deduced amino acid sequence (Fig. 1) predicts a protein of 693 residues, suggesting an unglycosylated molecular weight of ≈70,000. This is consistent with size estimates from studies in which the dopamine transporter was labeled with photoaffinity probes and analyzed by SDS/polyacrylamide gel electrophoresis (13, 27).

The nucleotide and derived amino acid sequences, as well as the predicted structure, are very similar to those of the rat GABA (16), human norepinephrine (18), and rat serotonin (36) transporters. After sequence alignment there is 40%, 66%, and 47% amino acid identity, respectively, and the similarity increases to 63%, 80%, and 69% when conservative amino acid substitutions are considered. Hydrophobicity analysis using the algorithm of Kyte and Doolittle (28) generates a profile virtually superimposable with the other cloned neurotransmitter transporters, and suggests 12–13 possible membrane-spanning domains, as previously described (16, 18). Three consensus sites for N-linked glycosylation are present in a region that may be a large extracellular loop; two or three such sites are present in the same region of the other neurotransmitter transporters. Potential

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DAT ..............MSEGRCSVAHMSSVVAPAKEANAMGP.......KAVELVLVKEQNGVQLTNSTLLNPPQSPTEA 80
Net ....................MLLARMNPQVQPENNGADTGPEQPLRARKTAELLVVKERNGVQ....CLLAPRDG..DA
Htt METTPLNSQKVLSECKDREDCQENGVLQKGVPTTADRAEPSQISNGYSAVPSTSAGDEASHSIPAATTTLVAE....IRQ
Gat ............................MATDNSKVADGQISTE.........VSEAPVASDKPKTLVVKVQKKAGDL

DAT QDRETWSKKADFLLSVIGFAVDLANVWRFPYLCYKNGGGAFLVPYLFFMVVAGVPLFYMELALGQFNREGAAGVW.KICP 160
Net QPRETWGKKIDFLLSVVGFAVDLANVWRFPYLCYKNGGGAFLIPYTLFLIIAGMPLFYMELALGQYNREGAATVW.KICP
Htt GERETWGKKMDFLLSVIGYAVDLGNIWRFPYICYQNGGGAFLLPYTIMAIFGGIPLFYMELALGQYHRNGCISIWRKICP
Gat PDRDTWKGRFDFLMSCVGYAIGLGNVWRFPYLCGKNGGGAFLIPYFLTLIFAGVPLFLLECSLGQYTSIGGLRVW.KLAP

DAT ILRGVGYTAILISLYIGFFYNVIIAWALHYLLSSFTTELPWTHCNHSWNSPRCSDARAPNASSGPNGTS....RTTPAAE 240
Net FFKGVGYAVILIALYVGFYYNVIIAWSLYYLFSSFTLNLPWTDCGHTWNSPNCTDPKLLNGSVLGNHTKYSKYKFTPAAE
Htt IFKGIGYAICIIAFYIASYYNTIIAWALYYLISSLTDRLPWTSCTNSWNTGNCTN......YFAQDNITWTLHSTSPAEE
Gat MFKGVGLAAAVLSFWLNIYYIVIISWAIYYLYNSFTTTLPWKQCDNPWNTDRC.........FSNYSLVNTTNMTSAVVE

DAT YFERGVLHLHESQGIDDLGPPRWQLTSCLVLVIVLLYFSLWKGVKTSGKVVWITATMPYVVLFALLLRGITLPGAVDAIR 320
Net FYERGVLHLHESSGIHDIGLPQWQLLLCLMVVVIVLYFSLWKGVKTSGKVVWITATLPYFVLFVLLVHGVTLPGASNGIN
Htt FYLRHVLQIHQSKGLQDLGTISWQLTLCIVLIFTVIYFSIWKGVKTSGKVVWVTATFPYIVLSVLLVRGATLPGAWRGVV
Gat FWERNMHQMTD..GLDKPGQIRWPLAITLAIAWVLVYFCIWKGVGWTGKVVYFSATYPYIMLIILFFRGVTLPGAKEGIL

DAT AYLSVDFHRLCEASVWIDAAIQICFSLGVGLGVLIAFSSYNKFTNNCYRDAIITTSVNSLTSFSSGFVVFSFLGYMAQKH 400
Net AYLHIDFYRLKEATVWIDAATQIFFSLGAGFGVLIAFASYNKFDNNCYRDALLTSSINCITSFVSGFAIFSILGYMAHEH
Htt FYLKPNWQKLLETGVWVDAAAQIFFSLGPGFGVLLAFASYNKFNNNCYQDALVTSVVNCMTSFVSGFVIFTVLGYMAEMR
Gat FYITPNFRKLSDSEVWLDAATQIFFSYGLGLGSLIALGSYNSFHNNVYRDSIIVCCINSCTSMFAGFVIFSIVGFMGHVT

DAT SVPIGDVAKD.GPGLIFIIYPEALATLPLSSVWAVVFFVMLLTLGIDSAMGGMESVITGLADEF.QLLHRHRELFTLLVV 480
Net KVNIEDVATE.GAGLVFILYPEAISTLSGSTFWAVVFFVMLLALGLDSSMGGMEAVITGLADDF.QVLKRHRKLFTFGVT
Htt NEDVSEVAKDAGPSLLFITYAEAIGNMPASTFFAIIFFLMLITLGLDSTFAGLEGVITAVLDEFPHIWAKRREWFVLIVV
Gat KRSIADVA.ASGRGLAFLAYPEAVTQLPISPLWAILFFSMLLMLGIDSQFCTVEGFITALVDEYPRLLRNRRELFIAAVC

DAT LATFLLSLFCVTNGGIYVFTLLDHF.AAGTSILFGVLMEVIGVAWFYGVWQFSDDIKQMTGRRPSLYWRLCWKFVSPCFL 560
Net FSTFLLALFCITKGGIYVLTLLDTF.AAGTSILFAVLMEAIGVSWFYGVDRFSNDIQQMMGFRPGLYWRLCWKFVSPAFL
Htt ITCVLGSLLTLTSGGAYVVTLLEEY.ATGPAVLTVALIEAVAVSWFYGITQFCSDVKEMLGF.SGMVWRICWVAISPLFL
Gat IVSYLIGLSNITQGGIYVFKLFDYYSASGMSLLFLVFFECVSISWFYGVNRFYDNIQEMVGSRPCIWWKLCWSFFTPIIV

DAT LFVVVVSIATFRPPHYGAYVFPEWATALGWAIAASSMSVVPIYAAYKLCSLPGSSREKLAYAITPETEHGRVDSGGGAPV 640
Net LFVVVVSIINFKPLTYDDYIFPPWANWVGWGIALSSMVLVPIYVIYKFLSTQGSLWERLAYGITPENEHHLVA.......
Htt LFIICSFLMSPPQLRLFQYNYPHWSIVLGYCIGMSSVICIPTYIIYRLISTPGTLKERIIKSITPETP...TEIRVGHPH
Gat AGVFLFSAVQMTPLTMGSYVFPKWGQGVGWLMALSSMVLIPGYMAYMFLTLKGSLKQRLQVMIQPSEDIVRPENGPEQPQ

DAT HAPPLARGVGRWRKRKSCWVPSRGPGRGGPPTPSPRLAGHTRAFPWTGAPPVPRELTPPSTCRCVPPLVCAHPAVESTGL 720
Net .....QRDIRQFQLQHWLAI
Htt ECCVTHPGRGHLFPATSLSSEKPTGLLL
Gat AGSSASKEAYI

DAT CSVY 725
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FIG. 1. Deduced amino acid sequence of the dopamine transporter (DAT) compared with those of the norepinephrine transporter (Net; ref. 18), serotonin transporter (Htt; ref. 36), and GABA transporter (Gat; ref. 16). Gaps have been added to obtain good alignment. Positions at which three or four of the amino acids are identical are shaded. Predicted transmembrane domains are underlined. Potential N-linked glycosylation sites (^) and sites for phosphorylation by cAMP-dependent protein kinase (★) and protein kinase C (▲) on the dopamine transporter are indicated.

sites for phosphorylation by cyclic AMP-dependent protein kinase are present on predicted intracellular domains near the carboxyl terminus of the DA transporter. There are also several possible sites for phosphorylation by protein kinase C (see Fig. 1).

An antisense oligonucleotide probe for *in situ* hybridization histochemistry was synthesized using the sequence from a region of the dopamine transporter cDNA with minimal similarity to the other cloned neurotransmitter transporters. There was strong hybridization of this probe to discrete cells in the bovine substantia nigra in areas corresponding to the A9 and A10 cell groups (Fig. 2 *Upper*) (2). There is striking correlation between this pattern of hybridization and that seen on an adjacent section probed with an oligonucleotide directed at tyrosine hydroxylase mRNA (Fig. 2 *Lower*). There was no detectable hybridization of the dopamine transporter probe to serotonin-containing cells in the raphe nuclei or to norepinephrine-containing cells in the locus coeruleus (data not shown), which were also labeled by the probe for tyrosine hydroxylase mRNA. Thus, the cDNA cloned does not encode either the bovine serotonin or the norepinephrine transporter. There is a significant GABA-containing cell population in the substantia nigra pars reticulata (29); however, the absence of hybridization in the caudate and elsewhere demonstrate that our putative dopamine transporter cDNA does not encode a GABA transporter. (A larger cDNA that hybridized less strongly on the original Southern blots of the library may be the bovine GABA transporter or another member of this family.) Under the hybridization and exposure conditions used there was no detectable labeling of cells in the tuberal region of the hypothalamus. While this area is known to contain dopaminergic neurons, there may have been too few to visualize or they may contain less dopamine transporter mRNA than do neurons in the substantia nigra. There have been reports, however, that the kinetics of dopamine uptake in the median eminence, posterior pituitary, and hypothalamus and in the striatum are different (8), implying that different transporters may exist.

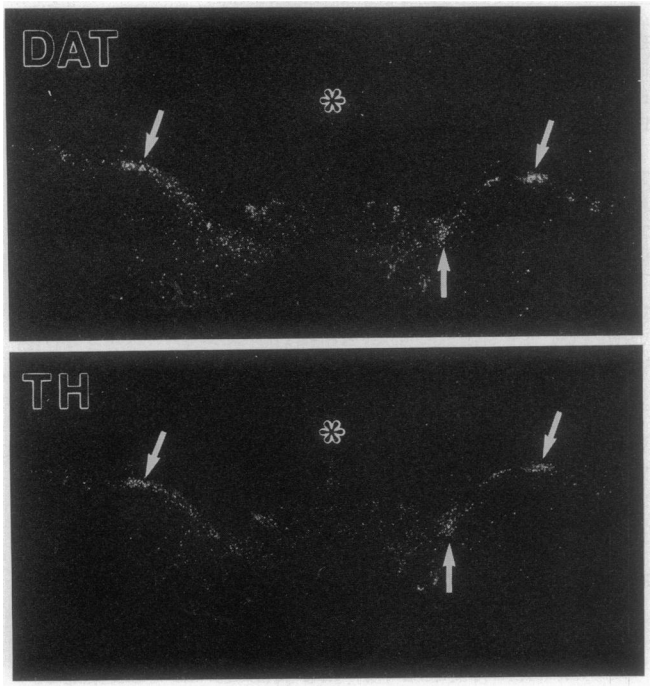

FIG. 2. *In situ* hybridization of dopamine transporter (DAT) (*Upper*) and tyrosine hydroxylase (TH) (*Lower*) oligonucleotide probes to 12-μm sections of bovine substantia nigra. Arrows indicate the same groups of neurons in the two adjacent sections; midline is indicated by the asterisk. An 8-day exposure on Hyperfilm-β max (Amersham) was printed as a negative (developed grains are white).

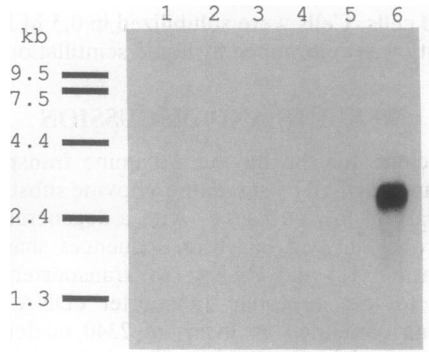

FIG. 3. Hybridization of the dopamine transporter oligonucleotide probe to bovine RNA. Approximately 6 μg of once-selected poly(A)$^+$ RNA from the adrenal medulla (lane 1), spleen (lane 2), hypothalmus (lane 3), cerebellum (lane 4), and caudate (lane 5), and 1.5 μg from the substantia nigra (lane 6) were loaded onto the gel. The blot was exposed overnight to X-AR5 film (Kodak) with an intensifying screen. kb, Kilobases.

The oligonucleotide used for *in situ* hybridization was labeled with ^{32}P and used to probe blots of RNA from several bovine tissues (Fig. 3). A single major band of ≈3.0 kb was present in RNA from the substantia nigra. Much less intense bands of 4.0 and 5.8 kb were also detected in the substantia nigra sample. After prolonged exposure a faint band of approximately the same size as the major band in the substantia nigra was seen in the sample from the hypothalmus. No additional bands were detected when a 1-kb fragment of the dopamine transporter cDNA was used as a probe (data not shown). Probing a blot of human substantia nigra RNA revealed a single band of ≈4.5 kb (data not shown).

The dopamine transporter was expressed in tissue culture cells by simultaneously introducing a recombinant vaccinia virus encoding bacteriophage SP6 RNA polymerase and the dopamine transporter cDNA. These cells accumulated [^{3}H]dopamine in a dose-dependent manner with a K_m of 31.5 μM, which is significantly higher than that for the rat and human dopamine transporters (8). Inhibition constants for a series of compounds were determined from displacement curves (Table 1) (30). GBR12909, a highly specific inhibitor of the dopamine transporter (31, 32), blocked [^{3}H]dopamine accumulation much more potently than did desipramine, which is more specific for the norepinephrine uptake system (33), or fluoxetine (34), which is more specific for the serotonin transporter. Cocaine and amphetamine, which appear to act at several monoamine reuptake sites, potently inhibited dopamine uptake. These pharmacological data con-

Table 1. Inhibition of [^{3}H]dopamine uptake by CV-1 cells expressing the putative dopamine transporter

Inhibitor	K_i, nM
Mazindol	51
GBR12909	52
Cocaine	244
Amphetamine	386
Nomifensine	536
Imipramine	1670
Fluoxetine	2000
Desipramine	7800
Bupropion	2740
MDMA*	5800

The following drugs did not inhibit [^{3}H]dopamine uptake at 10 μM: norepinephrine, reserpine, serotonin, L-dopa.
*3,4-Methylenedioxymethamphetamine.

firm that this cDNA clone encodes the bovine dopamine transporter.

In this study weak inhibition of [^{3}H]dopamine uptake by norepinephrine was observed. This is in contrast to the relatively similar potencies of dopamine and norepinephrine in inhibiting [^{3}H]norepinephrine transport by the cloned norepinephrine transporter (18). With the availability of homogeneous preparations expressed in tissue culture cells, pharmacological studies of truly single transporters have become possible. This may lead to the development of more specific, clinically useful drugs.

A possibility raised by the deduced peptide sequence of the dopamine transporter is regulation by cyclic AMP (or cyclic GMP)-dependent protein kinase, since potential sites for phosphorylation by these enzymes are present on domains predicted to be intracellular. Stimulation of dopamine accumulation by dibutyryl cyclic AMP and forskolin in tissue culture cells has recently been reported (35). The cloned transporter presents an opportunity for detailed study of its interaction with other proteins and with second-messenger systems and the possibility to identify regulatory influences on this important component of synaptic physiology.

Note Added in Proof. While this paper was in press, two descriptions of the rat dopamine transporter were published (37, 38).

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1. Ferland, L., Labrie, F., Cusan, L., Dupont, A., Lepine, J., Beaulieu, M., Denizeau, F. & Lemay, A. (1980) in *The Endocrine Functions of the Brain*, ed. Motta, M. (Raven, New York), pp. 271–296.
2. Björklund, A. & Lindvall, O. (1984) in *Handbook of Chemical Neuroanatomy*, eds. Bjorklund, A. & Hökfelt, T. (Elsevier, Amsterdam), pp. 55–122.
3. Iversen, S. (1977) in *Handbook of Psychopharmacology*, eds. Iversen, L., Iversen, S. D. & Snyder, S. H. (Plenum, New York), pp. 333–384.
4. Le Moal, M. & Simon, H. (1991) *Physiol. Rev.* **71**, 155–234.
5. Axelrod, J. (1971) *Science* **173**, 598–606.
6. Hertting, G., Axelrod, J., Kopin, I. J. & Whitby, L. G. (1961) *Nature (London)* **189**, 66–68.
7. Iversen, L. L. (1973) *Br. Med. Bull.* **29**, 130–135.
8. Horn, A. S. (1990) *Prog. Neurobiol.* **34**, 387–400.
9. Raiteri, M., Cerrito, F., Cervoni, A. M., del Carmine, R., Ribera, M. T. & Levi, G. (1978) in *Advances in Biochemical Psychomarmacology*, ed. Roberts, P. J. (Raven, New York), pp. 35–56.
10. Javitch, J. A., Strittmatter, S. M. & Snyder, S. H. (1985) *J. Neurosci.* **5**, 1513–1521.
11. Javitch, J. A., Blaustein, R. O. & Snyder, S. H. (1984) *Mol. Pharmacol.* **26**, 35–44.
12. Horn, A. S. (1978) in *Advances in Biochemical Psychomarmacology*, ed. Roberts, P. J. (Raven, New York), pp. 25–34.
13. Grigoriadis, D. E., Wilson, A. A., Lew, R., Sharkey, J. S. & Kuhar, M. J. (1989) *J. Neurosci.* **9**, 2664–2670.
14. Johanson, C.-E. & Fischman, M. W. (1989) *Pharmacol. Rev.* **41**, 3–52.
15. Ritz, M. C., Lamb, R. J., Goldberg, S. R. & Kuhar, M. J. (1987) *Science* **237**, 1219–1223.
16. Guastella, J., Nelson, N., Nelson, H., Czyzyk, L., Keynan, S., Miedel, M. C., Davidson, N., Lester, H. A. & Kanner, B. I. (1990) *Science* **249**, 1303–1306.
17. Nelson, H., Mandiyan, S. & Nelson, N. (1990) *FEBS Lett.* **269**, 181–184.
18. Pacholczyk, T., Blakely, R. D. & Amara, S. G. (1991) *Nature (London)* **350**, 350–354.
19. Okayama, H., Kawaichi, M., Brownstein, M., Lee, F., Yokota, T. & Arai, K. (1987) *Methods Enzymol.* **154**, 3–28.
20. Bonner, T. I., Buckley, N. J., Young, A. C. & Brann, M. R. (1987) *Science* **237**, 527–532.
21. Bonner, T., Affolter, H.-U., Young, A. C. & Young, W. S., III (1987) *Mol. Brain Res.* **2**, 243–249.
22. Devereux, J., Haeberli, P. & Smithies, O. (1984) *Nucleic Acids Res.* **12**, 387–395.
23. Saadat, S., Stehle, A. D., Lamouroux, A., Mallet, J. & Thoenen, H. (1988) *J. Neurochem.* **51**, 572–578.
24. Young, W. S., III, Mezey, E. & Siegel, R. E. (1986) *Mol. Brain Res.* **1**, 231–241.
25. Fuerst, T. R., Niles, E. G., Studier, F. W. & Moss, B. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 8122–8126.
26. Fuerst, T. R., Earl, P. L. & Moss, B. (1987) *Mol. Cell. Biol.* **7**, 2538–2444.
27. Berger, S. P., Martenson, R. E., Laing, P., Thurkauf, A., Decosta, B., Rice, K. & Paul, S. M. (1991) *Mol. Pharmacol.* **39**, 429–435.
28. Kyte, J. & Doolittle, R. F. (1982) *J. Mol. Biol.* **157**, 105–132.
29. Mugnaini, E. & Oertel, W. H. (1985) in *Handbook of Chemical Neuroanatomy*, eds. Bjorklund, A. & Hokfelt, T. (Elsevier, Amsterdam), pp. 436–622.
30. Cheng, Y. & Prusoff, W. H. (1973) *Biochem. Pharmacol.* **22**, 3099–3108.
31. Andersen, P. H. (1987) *J. Neurochem.* **48**, 1887–1896.
32. Andersen, P. H. (1989) *Eur. J. Pharmacol.* **166**, 493–504.
33. Horn, A. S. (1976) in *The Mechanism of Neuronal and Extraneuronal Transport of Catecholamines*, ed. Paton, D. M. (Raven, New York), pp. 195–214.
34. Lemberger, L. H., Rowe, H., Carmichael, R., Crabtree, R., Horng, J. S. & Bymaster, F. (1978) *Clin. Pharmacol. Ther.* **23**, 421–429.
35. Kadowaki, K., Hirota, K., Koike, K., Ohmichi, M., Kiyama, H., Miyake, A. & Tanizawa, O. (1990) *Neuroendocrinology* **52**, 256–261.
36. Hoffman, B. J., Mezey, E. & Brownstein, M. J. (1991) *Science* **254**, 579–580.
37. Shimada, S., Kitayama, S., Lin, C.-L., Patel, A., Nanthakumar, E., Gregor, P., Kuhar, M. & Uhl, G. (1991) *Science* **254**, 576–578.
38. Kilty, J. E., Lorang, D. & Amara, S. G. (1991) *Science* **254**, 578–579.