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Stimulation of high-affinity adenosine A_2 receptors decreases the affinity of dopamine D_2 receptors in rat striatal membranes

{2-[*p*-(2-carboxyethyl)phenethylamino]-5′-*N*-ethylcarboxamidoadenosine/GTP/*N*-propylnorapomorphine/raclopride/receptor–receptor interactions}

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ABSTRACT **Since high-affinity adenosine A_2 receptors (A_{2a}) are localized exclusively in dopamine-rich regions in the central nervous system and mediate inhibition of locomotor activity, we have examined the effect of A_{2a} receptor activation on D_1 and D_2 receptor binding in membrane preparations of the rat striatum. The A_{2a} agonist 2-[*p*-(2-carboxyethyl)phenethylamino]-5′-*N*-ethylcarboxamidoadenosine (CGS 21680) increased the K_d of the dopamine D_2 agonist L-(−)-*N*-[^{3}H]propylnorapomorphine without affecting the B_{max}. The increase in K_d was maximal (40%) at 30 nM CGS 21680. CGS 21680 (30 nM) decreased the dopamine-induced inhibition of [^{3}H]raclopride (a D_2 antagonist) binding due to an increase (about 3-fold) in K_H and K_L, the dissociation constants of high- and low-affinity binding sites. The effects of CGS 21680 were antagonized by the adenosine antagonist 8-phenyltheophylline (10 μM). (−)-N^6-(2-Phenylisopropyl)adenosine produced an effect similar to that of CGS 21680, provided the concentration used was high enough to stimulate A_{2a} receptors (300 nM). GTP (50 μM) also decreased the dopamine-induced inhibition of [^{3}H]raclopride binding but, in contrast to CGS 21680, GTP decreased the proportion of D_2 receptors in the high-affinity state. CGS 21680 (30 nM) did not affect the K_d or B_{max} of [^{3}H]raclopride and failed to affect ligand binding to D_1 receptors. Thus, stimulation of A_{2a} receptors potently reduces the affinity of D_2 agonist binding sites within the plasma membrane of striatal neurons. This A_{2a}–D_2 interaction may underlie the neuroleptic-like actions of adenosine agonists and the enhancing effects of adenosine antagonists, such as caffeine, on locomotor activity.**

Adenosine has been shown to function as a neuromodulator in many areas of the mammalian central nervous system (1–3). These actions of adenosine are mediated by receptors that can be subdivided into A_1 and A_2 subtypes based on relative agonist and antagonist potencies (4, 5). A_1 activation inhibits and A_2 activation stimulates adenylate cyclase (4, 6, 7). The A_2 receptors have been further subclassified into high-affinity (A_{2a}) and low-affinity (A_{2b}) receptors, based on agonist potencies with regard to adenylate cyclase activation (4) and receptor binding (5). The A_{2b} receptors are widely distributed in the brain and mediate the stimulatory action of high concentrations of adenosine agonists on cAMP formation, which could affect dopamine release and synthesis (8, 9). In contrast, A_{2a} receptors are exclusively localized to dopamine-innervated areas of the central nervous system (10, 11), with a postsynaptic distribution (12) similar to that of postsynaptic D_1 and D_2 receptors (13). By using *in situ* hybridization the recently cloned A_{2a} receptors (14) have been found to be localized to striatal medium-sized neurons (15).

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Adenosine agonists inhibit, whereas adenosine antagonists, including caffeine, enhance spontaneous (16, 17) and dopamine-induced locomotor activity (16, 18–20). The potencies of adenosine agonists in producing hypomotility correlate with their affinities for A_{2a} adenosine receptors (21, 22), suggesting that A_{2a} receptors mediate most of the behavioral effects of adenosine agonists. The hypomotility induced by adenosine agonists resembles that induced by classical neuroleptics (22), which act by blocking postsynaptic D_2 receptors (23). In fact, behavioral evidence for a negative interaction between postsynaptic A_{2a} and D_2 receptors has recently been obtained using acutely reserpinized mice (24, 25). Since activation of postsynaptic D_2 receptors seems to be a necessary step for locomotor behavior, a negative interaction between postsynaptic A_{2a} and D_2 receptors could explain the hypomotility induced by adenosine agonists and the enhancement of locomotor activity induced by adenosine antagonists, including caffeine (24, 25).

In contrast to the A_{2a} receptor, the D_2 receptor mediates an inhibition of adenylate cyclase (26). However, the stimulatory activity of A_{2a} receptors on adenylate cyclase cannot simply explain the counteractive action of A_{2a} stimulation on D_2-mediated locomotor activity since D_1 receptors, which also stimulate adenylate cyclase, potentiate D_2-mediated locomotor activity (24). One possibility could be the existence of a more direct interaction between A_{2a} and D_2 receptors. Earlier studies have shown that L-glutamate (27), SCH 23390 (28), and neuropeptides such as neurotensin (29–31, 44) and cholecystokinins 4 and -8 (32) can modulate the binding characteristics of striatal D_2 agonist binding sites by means of intramembrane interactions. To investigate the existence of an intramembrane interaction between A_{2a} and D_2 receptors, we have examined whether adenosine analogs such as the recently developed selective A_{2a} agonist 2-[*p*-(2-carboxyethyl)phenethylamino]-5′-*N*-ethylcarboxamidoadenosine (CGS 21680) (33–35) can modulate the binding characteristics of dopamine D_2 receptors in membrane preparations of rat striatum. It was found that, in rat striatal membranes, the stimulation of A_{2a} receptors decreases the affinity of D_2 agonist binding.

MATERIALS AND METHODS

Animals. Male Sprague–Dawley rats (body weight, 200–250 g; Alab, Stockholm) were kept under regular lighting conditions (lights on at 06:00 and off at 20:00) in a tempera-

Abbreviations: CGS 21680, 2-[*p*-(2-carboxyethyl)phenethylamino]-5′-*N*-ethylcarboxamidoadenosine; NPA, L-(−)-*N*-propylnorapomorphine; R-PIA, (−)-N^6-(2-phenylisopropyl)adenosine; 8-PT, 8-phenyltheophylline; SCH 23390, (*R*)-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride.

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ture-controlled environment and had free access to food pellets and tap water. The rats were decapitated with a guilliotine, and the brain was rapidly removed and placed on ice. The brain was partially cut in the midline and the neostriatum was removed with a sharp forceps.

Saturation Experiments with L-(−)-*N*-[^{3}H]Propylnorapomorphine ([^{3}H]NPA). The tissue was weighed, introduced in polypropylene vials, and sonicated for 30 s in ice-cold Tris·HCl buffer (50 mM, pH 7.6) containing 0.01% L-(+)-ascorbic acid and 1 mM EDTA. Tris buffer (as above) was added and the homogenate was centrifuged for 10 min at 45,000 × *g* (Sorvall SS-34 rotor; RC-5B centrifuge, DuPont Instruments, Sorvall Division). The supernatant was discarded and the membrane pellet was resuspended by sonication in Tris buffer and preincubated for 30 min at 37°C. This preincubation was followed by another centrifugation for 10 min at 45,000 × *g*. The pellet was resuspended in Tris buffer (final concentration of membranes, 2.5 mg of wet weight per 0.5 ml of incubation medium), and saturation curves with 10 concentrations (0.05–2 nM) of the D_2 agonist [^{3}H]NPA (2.0 TBq/mmol; NEN) (30) were determined by incubation for 30 min at 25°C with or without the presence of the various drugs under study [CGS 21680, 8-phenyltheophylline (8-PT), GTP]. Nonspecific binding was defined as the binding in the presence of raclopride (1 μM) (Astra Läkemedel, Södertälje, Sweden) (36). The incubation was stopped by washing the membranes three times with 5 ml of ice-cold Tris buffer over a Whatman GF/B filter (Millipore) under reduced pressure. The radioactivity content of the filters was detected by liquid scintillation spectroscopy. Protein contents were determined with bovine serum albumin as a standard (37).

Saturation Experiments with [^{3}H]Raclopride. The tissue was prepared as described above, in the same buffer as for [^{3}H]NPA binding but also including $MgCl_2$ (5 mM). Saturation experiments with 10 concentrations (0.5–15 nM) were performed by incubation with [^{3}H]raclopride (1.5 TBq/mmol, Astra Läkemedel, Södertälje, Sweden) (36) for 30 min at room temperature in the presence or absence of CGS 21680. Nonspecific binding was defined as the binding in the presence of (+)-butaclamol (1 μM).

Competition Experiments with Dopamine Versus [^{3}H]Raclopride. The tissue was prepared as in saturation experiments with [^{3}H]raclopride. Competitive inhibition experiments with 20 single concentrations (10 pM–1 mM) of dopamine were performed by incubation with 2 nM [^{3}H]raclopride for 30 min at room temperature in the presence or absence of the different drugs under study [CGS 21680, 8-PT, GTP, (−)-N^6-(2-phenylisopropyl)adenosine (R-PIA)].

Competition Experiments with Dopamine Versus (*R*)-(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-[*N*-methyl-^{3}H]benzazepine Hydrochloride, ([^{3}H]SCH 23390). The tissue was prepared as described above, but without the preincubation, in 50 mM Tris·HCl buffer (pH 7.4) containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$. Competition experiments with 20 concentrations (10 pM–1 mM) of dopamine were performed by incubating the membranes with [^{3}H]SCH 23390 (2.6 TBq/mmol; NEN) (38) for 15 min at 37°C in the presence or absence of the different drugs under study (CGS 21680, GTP).

Data Analysis. Data from saturation experiments were analyzed by nonlinear regression analysis of the raw data for the determination of K_d and B_{max} values using a computer program kindly provided by Stéphane Swillens (Institut de Recherche Interdisciplinaire, Université Libre de Bruxelles, Campus Erasme, Brussels, Belgium). Linear fitting of Scatchard plots from the same data yielded similar estimates. Data from competition experiments were analyzed by the LIGAND program (39). The specific binding and affinity for each affinity state were determined by computerized nonlinear iterative least-square regression for each curve separately. The amount of nonspecific binding (about 0.5% of the free ligand) was calculated by extrapolation of the displacement curve. K_d, B_{max}, pseudo-Hill coefficients, K_H and K_L,¶ as well as the proportion of receptors in the high-affinity state (R_H) were analyzed by Student's paired *t* test or by single-factor repeated measures analysis of variance followed by Fisher's protected least-square difference method. To achieve homogeneity of variance and allow parametric statistical analysis, K_H and K_L values were logarithmically transformed (40).

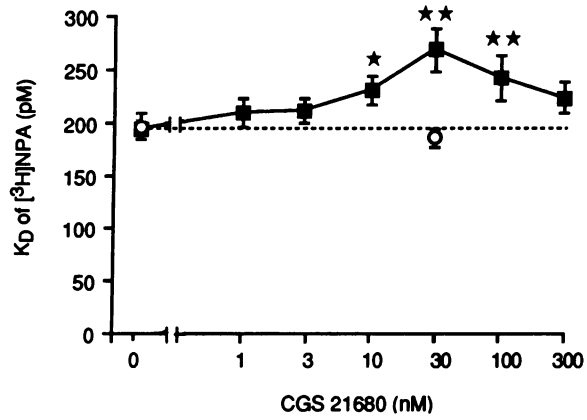

FIG. 1. Effect of increasing concentration of CGS 21680 on K_d values of [^{3}H]NPA binding in rat striatal membranes. Membranes were incubated with [^{3}H]NPA and the indicated concentrations of CGS 21680 alone (■) or in the presence (○) of 8-PT (10 μM) for 30 min at room temperature. K_d and B_{max} were calculated by nonlinear regression from saturation curves using 10 concentrations of [^{3}H]NPA, with raclopride (1 μM) for the determination of nonspecific binding. Data show the mean ± SEM of nine separate experiments (four experiments for 8-PT). ★$P < 0.05$ and ★★$P < 0.01$ against control. The B_{max} values, 193 ± 7, 214 ± 16, and 217 ± 8 fmol/mg of protein for control, CGS 21680 (30 nM), and 8-PT (10 μM), respectively, were not significantly affected.

RESULTS

Saturation Experiments with [^{3}H]NPA. CGS 21680 (10–100 nM) increased the K_d of [^{3}H]NPA binding sites, without affecting the B_{max} (Figs. 1 and 2). The increase in K_d was maximal (about 40%) at 30 nM CGS 21680 and disappeared at 300 nM. 8-PT (10 μM), which by itself did not produce any change in [^{3}H]NPA binding, completely antagonized the effect of CGS 21680 (Fig. 1). GTP (50 μM) increased the K_d of [^{3}H]NPA by 53% ± 17% without significantly affecting the B_{max} (data not shown).

Saturation Experiments with [^{3}H]Raclopride. CGS 21680 (30 nM) did not affect the affinity or the density of [^{3}H]raclopride binding sites (4.95 ± 0.49 nM and 360 ± 31 fmol/mg of protein for control versus 5.06 ± 0.19 nM and 364 ± 25 fmol/mg of protein for CGS 21680; four paired experiments).

Competition Experiments with Dopamine Versus [^{3}H]Raclopride. Competition curves with dopamine versus [^{3}H]raclopride showed a significantly better fit for two binding sites than for one (pseudo-Hill coefficient, around 0.5–0.6; data not shown). CGS 21680 (30 nM but not 300 nM) significantly decreased the dopamine-induced inhibition of [^{3}H]raclopride binding, by increasing by about 3-fold K_H and K_L, without affecting R_H (Fig. 3; Table 1). These increases were completely antagonized by 8-PT (10 μM), which by itself did not produce any changes in K_H and K_L (Table 1).

¶K_H and K_L are the dissociation constants of high-affinity and low-affinity binding sites presented as antilogarithms (geometric mean and 95% confidence limits of the geometric mean) of the logarithmic-transformed data used for statistical analysis (40).

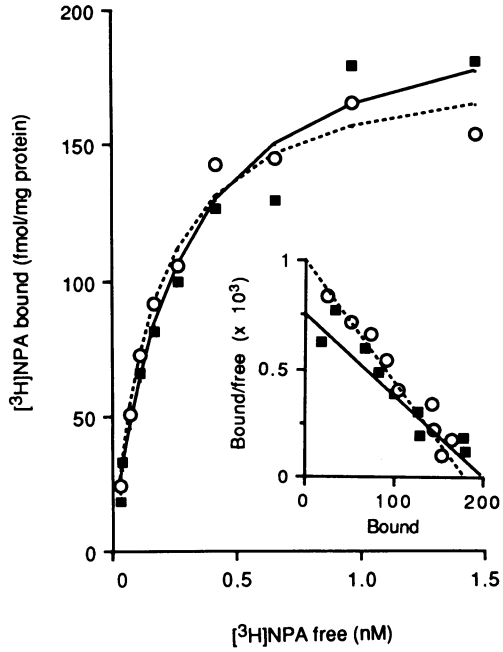

FIG. 2. Representative saturation curves showing the effect of CGS 21680 on [^{3}H]NPA binding in rat striatal membranes. Membranes were incubated with CGS 21680 (30 nM) and [^{3}H]NPA for 30 min at room temperature. The K_d and B_{max} were 171 pM and 184 fmol/mg of protein for control (○) and 251 pM and 207 fmol/mg of protein in the presence of CGS 21680 (■) as calculated by nonlinear regression using raclopride (1 μM) for the determination of nonspecific binding. (*Inset*) Corresponding Scatchard plots of the saturation binding data.

In a concentration that selectively stimulates A_1 receptors, R-PIA (3 nM) did not affect the competition curves of dopamine versus [^{3}H]raclopride. However, in a concentration that activates A_{2a} receptors (300 nM), R-PIA produced an ≈3-fold increase in K_H and K_L, similar to CGS 21680 at 30 nM, without affecting R_H (Table 1).

GTP (50 μM) also decreased the dopamine-induced inhibition of [^{3}H]raclopride binding but, in contrast to CGS 21680, GTP reduced R_H, without affecting K_H or K_L (Table 1).

Competition Experiments with Dopamine Versus [^{3}H]SCH 23390. Competition curves with dopamine versus [^{3}H]SCH 23390 showed a significantly better fit for two binding sites than for one (pseudo-Hill coefficient, around 0.5–0.6; data not shown). CGS 21680 (30 nM) did not affect the dopamine-induced inhibition of [^{3}H]SCH 23390 binding, in contrast to GTP (50 μM), which decreased R_H (Table 2).

DISCUSSION

The present study has shown that stimulation of adenosine A_{2a} receptors causes a reduction in the affinity of dopamine D_2 agonist binding sites in membrane preparations from rat striatum. In concentrations at which they act on A_{2a} receptors, CGS 21680 (34, 35) and R-PIA (5, 34) affected K_H and K_L of D_2 agonist binding sites. The reduced affinity of D_2 agonist binding was observed by using the agonist [^{3}H]NPA, which selectively labels D_2 sites under the present conditions (30), and by displacing the specific D_2 antagonist [^{3}H]raclopride with dopamine. The increase in K_d (40%) of [^{3}H]NPA binding was lower than that observed in K_H and K_L (about 3-fold) for competition curves with dopamine versus [^{3}H]-raclopride. One possible explanation for this difference could be the fact that [^{3}H]NPA binds to the D_2 receptor with a very high affinity and therefore may be less sensitive to possible conformational changes of the D_2 agonist binding site.

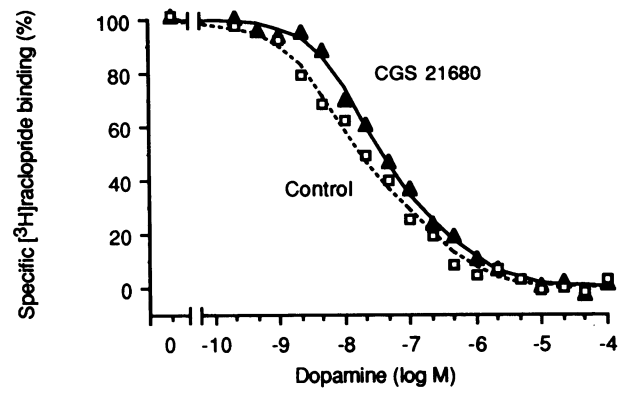

FIG. 3. Representative competitive-inhibition curves illustrating the effect of CGS 21680 (30 nM) on dopamine-induced inhibition of [^{3}H]raclopride binding in rat striatal membranes. By using the LIGAND program (39), the dissociation constants of high-affinity and low-affinity D_2 agonist binding (K_H and K_L) were estimated to 3.9 nM and 148 nM under control conditions and 11.8 nM and 363 nM in the presence of CGS 21680. The percentage of D_2 agonist binding sites (R_H) in the high-affinity state was 65% and 73%, respectively.

The similarity of action of CGS 21680 and of R-PIA (at high concentrations), which are structurally dissimilar agonists, and the fact that the effect of CGS 21680 on the D_2 agonist binding sites was completely antagonized by the adenosine receptor antagonist 8-PT indicate that the interaction is mediated by means of A_{2a} receptors and not by competitive binding to D_2 receptors, as is the case for classical neuroleptics (23). The A_{2a}–D_2 interaction seems to be specific, since stimulation of A_1 receptors with R-PIA at a concentration (3 nM) close to its K_d for A_1 receptors (5, 34) failed to affect D_2 agonist binding. In addition, stimulation of A_{2a} receptors with CGS 21680 (30 nM) failed to affect D_1 agonist binding sites.

Neither the affinity of D_2 antagonist binding nor the number of D_2 receptors was affected by CGS 21680, suggesting that it is the agonist binding to D_2 that is selectively affected. At 30 nM, CGS 21680 produced a reduction of D_2 agonist

Table 1. Competition experiments with dopamine versus [^{3}H]raclopride

Treatment	K_H, nM	K_L, nM	R_H, % of total
Control	3.00 (2.36–3.81)	265 (232–304)	66.2 ± 3.6
CGS 21680 (30 nM)	11.27 (8.36–15.21)**	977 (604–1581)*	70.8 ± 5.5
+8-PT (10 μM)	2.21 (1.66–2.94)	134 (95–189)	58.9 ± 4.9
8-PT (10 μM)	2.76 (1.75–4.36)	330 (238–456)	70.2 ± 4.0
CGS 21680 (300 nM)	7.05 (6.24–7.96)	340 (289–399)	72.0 ± 2.3
R-PIA (3 nM)	5.31 (3.61–7.82)	357 (249–513)	70.7 ± 6.4
R-PIA (300 nM)	12.65 (10.99–14.56)*	1096 (647–1857)**	77.7 ± 3.0
GTP (50 μM)	1.91 (1.18–3.09)	347 (257–468)	46.6 ± 9.9**

K_H and K_L are defined in *Materials and Methods*; the 95% confidence limits of the geometric mean are given in parentheses. R_H is the percentage of total specific binding sites in the high-affinity state shown as mean ± SEM. Each treatment is represented by four to eight separate experiments.
*$P < 0.05$ and **$P < 0.01$ against control.

Table 2. Competition experiments with dopamine versus [^{3}H]SCH 23390

Treatment	K_H, nM	K_L, nM	R_H, % of total
Control	33.9 (28.4–40.4)	1549 (1346–1782)	41.7 ± 4.3
CGS 21680 (30 nM)	24.5 (19.4–31.1)	2089 (1982–2203)	44.1 ± 2.2
GTP (50 μM)	41.2 (30.5–55.6)	1698 (1584–1905)	9.4 ± 2.2*

See legend to Table 1. Data represent six separate experiments. *$P < 0.05$ against control.

binding comparable to that induced by 50 μM GTP. However, the effect of A_{2a} receptor stimulation on D_2 agonist binding was different from that seen with GTP, which, in agreement with earlier results, converted some of the high-affinity binding sites to low-affinity binding sites without affecting their affinity constants (41, 42). These results suggest that the effect of A_{2a} receptors, which are coupled to G proteins (11), is mediated by a different mechanism than the effect of GTP. This mechanism may be similar to the G protein-independent mechanism mediating the neurotensin–D_2 interaction (31, 44), whereas the D_1–D_2 interaction seems to involve G proteins (28).

The concentration of CGS 21680 producing the maximal effect on D_2 agonist binding is close to its K_d value for the A_{2a} receptor. This indicates that the maximal effect on D_2 agonist binding is obtained when the A_{2a} receptor is stimulated by drugs close to their K_d values. At saturating concentrations of CGS 21680 (300 nM), the D_2 agonist binding was not significantly altered, as seen from the K_d value of [^{3}H]NPA binding and from the K_H and K_L of competition curves with dopamine versus [^{3}H]raclopride. This biphasic concentration–response curve is similar to that seen with neurotensin (31, 44) and may be due to a desensitization of the A_{2a} receptor that is taking place within the plasma membrane or the exhaustion of some essential endogenous factor. However, it differs from the monophasic decrease in D_2 agonist binding seen following treatment with neurotensin *in vivo* (43) or with direct D_2 antagonists.

In conclusion, the present study has revealed that stimulation of A_{2a} receptors potently reduces the affinity of D_2 agonist binding sites in striatal membranes. This interaction, which is the strongest receptor–receptor interaction at the binding site hitherto observed, could explain the neuroleptic-like effects of adenosine agonists and why adenosine antagonists, such as caffeine, enhance locomotor activity.

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1. Fredholm, B. B. & Hedqvist, P. (1980) *Biochem. Pharmacol.* **29**, 1635–1643.
2. Dunwiddie, T. V. (1985) *Int. Rev. Neurobiol.* **27**, 63–139.
3. Snyder, S. H. (1985) *Annu. Rev. Neurosci.* **8**, 103–124.
4. Daly, J. W., Butts-Lamb, P. & Padgett, W. (1983) *Cell. Mol. Neurobiol.* **1**, 69–80.
5. Bruns, R. F., Ku, G. H. & Pugsley, T. A. (1986) *Mol. Pharmacol.* **29**, 331–346.
6. van Calker, D., Möller, M. & Hamprecht, B. (1979) *J. Neurochem.* **33**, 999–1005.
7. Premont, J., Perez, M., Blanc, G., Tassin, J.-P., Thierry, H.-M., Hervé, D. & Bockaert, J. (1979) *Mol. Pharmacol.* **16**, 790–804.
8. Onali, P., Olianas, M. C. & Bunse, B. (1988) *Brain Res.* **456**, 302–309.
9. Booth, R. G. & Baldessarini, R. J. (1990) *Eur. J. Pharmacol.* **185**, 217–221.
10. Jarvis, M. F. & Williams, M. (1989) *Eur. J. Pharmacol.* **168**, 243–246.
11. Parkinson, F. E. & Fredholm, B. B. (1990) *Naunyn-Schmiedeberg's Arch. Pharmacol.* **342**, 85–89.
12. Alexander, S. P. & Reddington, M. (1989) *Neuroscience* **28**, 645–651.
13. Boyson, S. J., McGonigle, P. & Scatton, P. B. (1986) *J. Neurosci.* **6**, 3177–3182.
14. Maenhaut, C., Van Sande, J., Libert, F., Abramowicz, M., Parmentier, M., Vanderhaegen, J.-J., Dumont, J. E., Vassart, G. & Schiffman, S. (1990) *Biochem. Biophys. Res. Commun.* **173**, 1169–1178.
15. Schiffmann, S. N., Libert, F., Vassart, G., Dumont, J. E. & Vanderhaegen, J.-J. (1990) *Brain Res.* **519**, 333–337.
16. Waldeck, B. (1973) *J. Neural Trans.* **34**, 61–72.
17. Snyder, S. H., Katims, J. J., Annau, Z., Bruns, R. F. & Daly, J. W. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 3260–3264.
18. Fuxe, K. & Ungerstedt, U. (1974) *Med. Biol.* **52**, 48–54.
19. Green, R. D., Proudfit, H. K. & Yeung, S.-M. H. (1982) *Science* **218**, 58–61.
20. Fredholm, B. B., Fuxe, K. & Agnati, L. (1976) *Eur. J. Pharmacol.* **38**, 31–38.
21. Durcan, M. J. & Morgan, P. F. (1989) *Eur. J. Pharmacol.* **168**, 285–290.
22. Heffner, T. G., Wiley, J. N., Williams, A. E., Bruns, R. F., Coughenour, L. L. & Downs, D. A. (1989) *Psychopharmacology* **98**, 31–37.
23. Seeman, P. (1987) *Synapse* **1**, 133–152.
24. Ferré, S., Herrera-Marschitz, M., Grabowska-Andén, M., Ungerstedt, U., Casas, M. & Andén, N.-E. (1991) *Eur. J. Pharmacol.* **192**, 25–30.
25. Ferré, S., Herrera-Marschitz, M., Grabowska-Andén, M., Casas, M., Ungerstedt, U. & Andén, N.-E. (1991) *Eur. J. Pharmacol.* **192**, 31–37.
26. Onali, P., Olianas, M. C. & Gessa, G. L. (1985) *Mol. Pharmacol.* **28**, 138–145.
27. Fuxe, K., Celani, M. F., Martire, M., Zini, I., Zoli, M. & Agnati, L. F. (1984) *Eur. J. Pharmacol.* **100**, 127–130.
28. Seeman, P., Niznik, H. B., Guan, H.-C., Booth, G. & Ulpian, C. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 10156–10160.
29. Agnati, L. F., Fuxe, K., Benfenati, F. & Battistini, N. (1983) *Acta Physiol. Scand.* **119**, 459–461.
30. von Euler, G., Fuxe, K., Benfenati, F., Hansson, T., Agnati, L. F. & Gustafsson, J.-Å. (1989) *Acta Physiol. Scand.* **135**, 443–448.
31. von Euler, G., van der Ploeg, I., Fredholm, B. B. & Fuxe, K. (1991) *J. Neurochem.* **56**, 178–183.
32. Agnati, L. F., Celani, M. F. & Fuxe, K. (1983) *Acta Physiol. Scand.* **118**, 79–81.
33. Hutchison, A. J., Webb, R. L., Oei, H. H., Ghai, G. R., Zimmerman, M. B. & Williams, M. (1989) *J. Pharmacol. Exp. Ther.* **251**, 47–55.
34. Jarvis, M. F., Schulz, R., Hutchison, A. J., Hoi Do, U., Sills, M. A. & Williams, M. (1989) *J. Pharmacol. Exp. Ther.* **251**, 888–893.
35. Lupica, C. R., Cass, W. A., Zahniser, N. R. & Dunwiddie, V. (1990) *J. Pharmacol. Exp. Ther.* **252**, 1134–1141.
36. Köhler, C., Hall, H., Ögren, S.-O. & Gawell, L. (1985) *Biochem. Pharmacol.* **34**, 2251–2259.
37. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
38. Hyttel, J. (1983) *Eur. J. Pharmacol.* **91**, 153–154.
39. Munson, P. J. & Rodbard, D. (1980) *Anal. Biochem.* **107**, 220–239.
40. Snedecor, G. W. & Cochran, W. G. (1980) in *Statistical Methods* (Iowa State Univ., Ames, IA), pp. 291–292.
41. Zahniser, N. R. & Molinoff, P. B. (1978) *Nature (London)* **275**, 453–455.
42. Creese, I., Sibley, D. R., Hamblin, M. W. & Leff, S. E. (1983) *Annu. Rev. Neurosci.* **6**, 43–71.
43. von Euler, G., Meister, B., Hökfelt, T., Eneroth P. & Fuxe, K. (1990) *Brain Res.* **531**, 253–262.
44. von Euler, G. (1991) *Brain Res.*, in press.