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Dopamine Release and Metabolism after Chronic Delivery of Selective or Nonselective Dopamine Autoreceptor Agonists

C. ANTHONY ALTAR,[1] BRET BERNER, PAULA BEALL, STEPHEN F. CARLSEN, and WILLIAM C. BOYAR

Pharmaceuticals Division, CIBA-GEIGY Corporation, Summit, New Jersey 07901 (C.A.A., W.C.B.), and Pharmaceuticals Division, CIBA-GEIGY Corporation, Ardsley, New York 10502 (B.B., P.B., S.F.C.)

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SUMMARY

The metabolism and release of dopamine by rat mesostriatal and mesolimbic dopamine neurons were determined after 2 or 14 days of subcutaneous administration via Alzet minipumps of a selective (CGS 15855A) or nonselective (apomorphine) dopamine autoreceptor agonist. Bioassays and high performance liquid chromatography assays showed that each drug was accurately delivered for the 2- and 14-day periods. CGS 15855A levels in the plasma and brain increased with increases in the daily dose given, although plasma levels of CGS 15855A at 14 days were less than those at 2 days for each dose. Striatal dopamine metabolism and release, assessed with dihydroxyphenylacetic acid and 3-methoxytyramine concentrations, respectively, were suppressed by 2-day treatments of 50–200 μg/day CGS 15855A or 250 μg/day apomorphine. These suppressions were potentiated by acute challenge with 1 mg/kg intraperitoneally of CGS 15855A or 2 mg/kg subcutaneously of apomorphine. In contrast, dopamine metabolism and release were unchanged after 14 days of administration of 40–400 μg/day of CGS 15855A or 250 μg/day of apomorphine, even when plasma levels of drug were as high as at 2 days. Dopamine release was decreased in only one of six groups 30 min after an additional acute injection of the agonist given for 14 days, whereas dopamine metabolism was decreased in five of six groups. Striatal dopamine levels were increased 20–57% after 14 but not 2 days of cgs 15855A followed by acute challenge with the vehicle or CGS 15855A injections. Thus, the responsiveness of dopamine neurons to the release-suppressing properties of dopamine autoreceptor agonists is mostly attenuated between 2 and 14 days of treatment. The ability of chronic CGS 15855A treatments to increase dopamine levels and, with acute CGS 15855A, to decrease DOPAC levels, indicates that autoreceptor control of dopamine metabolism is partly retained after chronic autoreceptor agonism.

Negative feedback mechanisms are essential to the diverse activities of brain dopamine neurons. The predominant feedback mechanisms that control these neurons are activated via the D_2 class of dopamine receptor. D_2 agonists decrease the electrical activity (1–3) and release (4), biosynthesis (5, 6), and catabolism (7, 8) of dopamine. Low doses of the D_2 agonist apomorphine, which confer selectivity for the autoreceptor, decrease locomotor activity of mice (9) and induce somnolence in humans (10). These and related findings indicate that a subsensitivity of dopamine autoreceptors may account for positive symptoms of schizophrenia (11) and the induction of psychosis in man by chronic amphetamine administration (12). Unfortunately, the predicted antipsychotic efficacy of apomorphine and *N*-(*n*-propyl)norapomorphine administration (13, 14) abated after only several days of continuous treatment (15, 16). Clearly, the treatment of psychotic patients with dopamine autoreceptor agonists is likely to succeed only when these compounds produce a sustained depression of dopaminergic neuronal function. Yet tolerance also appears to be a frequent result of chronic dopamine agonist administration to animals besides humans. For example, the electrophysiology of nigrostriatal (17) and mesolimbic (18) dopaminergic cell bodies reveals that daily SC injections of apomorphine or the dopamine-releasing agent *d*-amphetamine produce within 7–9 days a tolerance to the ability of amphetamine to block neuronal electrical impulses. Tolerance in these and other (19, 20) studies may have occurred for several reasons, including the use of relatively high doses of dopamine agonists, which also stimulate post-synaptic D_2 receptors, and the use of daily bolus administrations. Multiple injections of the highly selective autoreceptor agonist CGS 15855A (21–23) prolong for at least 3 hr a marked suppression of dopamine metabolism and release in the caudate-putamen (22). We tested here whether sustained SC delivery for 2 or 14 days of CGS 15855A or apomorphine produces a sustained suppression of dopamine metabolism and release. Acute challenges with the same agonist given for 2 or

[1] Present address: Pharmacological Sciences, Genentech, Inc., South San Francisco, CA 94080.

ABBREVIATIONS: SC, subcutaneous; CGS 15855A (±)-*trans*-1,3,4,4a,5,10b-hexahydro-4-propyl-2*H*-[1]benzopyrano[3,4-b]-pyridin-9-ol monohydrochloride; DOPAC, dihydroxyphenylacetic acid; HVA, homovanillic acid; 3-MT, 3-methoxytyramine; IP, intraperitoneal; GC-MS, gas chromatography-mass spectrometry; HPLC, high performance liquid chromatography.

14 days were also examined for the effects of sustained agonist delivery on the acute suppression of dopamine release or metabolism.

Dopamine metabolism was assessed with measurements of DOPAC and HVA (24). These acid metabolites, and particularly DOPAC, are sensitive indicators of the ability of dopamine agonists to suppress dopamine metabolism (7, 25). Dopamine release can be inferred from measured levels of 3-MT (24, 26), especially when release is decreased by dopamine agonists (22, 23, 25).

Materials and Methods

Male rats (160–180 g, Tac:SD; Taconic Farms, New York; n = 7–9 per group) were administered, via Alzet minipumps (Alza Corp., Palo Alto, CA) either the saline vehicle, CGS 15855A (CIBA-GEIGY Corp., Summit, NJ), or apomorphine·HCl (Merck Sharp and Dohme, West Point, PA) for 2 or 14 days. The vehicle consisted of sterile saline (0.9%) that contained 1.0% ascorbic acid to retard apomorphine oxidation (27).

For the 2-day study, CGS 15855A was dissolved in 0.9% saline to concentrations for administration at 50, 100, or 200 μg/day; apomorphine was dissolved in 1.0% ascorbate, 0.9% saline to achieve an administration of 250 μg/day. Model 2 ML2 pumps were used to deliver their contents for 2 weeks at 4.5 ± 0.2 μl/hr. Concentrations of CGS 15855A were selected for the 14-day study for administration of the drug at 40, 100, and 400 μg/day. These doses were based on the relative potencies of either drug in the *in vivo* γ-butyrolactone model for dopamine autoreceptor activity (ED_{50}, apomorphine = 0.56 mg/kg, IP; CGS 15855A = 0.16 mg/kg; Ref. 21) and the *in vivo* suppression of dopamine release assessed with 3-MT (approximate ED_{50}, apomorphine = 0.1 mg/kg IP; CGS 15855A = 0.4 mg/kg; Ref. 22). The filled pumps were presoaked for 24 hr in capped 50-ml centrifuge tubes containing 0.9% saline.

Each animal was anesthetized with diethyl ether. The abdomen was shaved and a 3-cm skin incision was made on the left side. A single Alzet pump was inserted with the dispensing cap facing rostrally. The incision was closed with three wound clips. All animals were housed singly in an animal vivarium maintained at 22 ± 2° and were given free access to food and tap water.

Drug stability and release studies—apomorphine. A bioassay was used to ascertain the chemical stability of apomorphine after 14 days of sustained minipump delivery. Each of three pumps was filled with a 1-mg/ml solution of apomorphine dissolved in 0.9% saline. The sealed pumps were placed in 25 ml of 0.9% saline in capped test tubes contained in a 37° shaker bath. The saline in the test tubes was replenished twice daily for 15 days, at which time the apomorphine solutions remaining in the pumps were combined and diluted with 0.5 mM ascorbic acid to a concentration of 1 mg/10 ml. Male mice (20 g; Tac:SW, Taconic Farms; n = 7–8 per group) were injected IP either with the 0.9% saline vehicle containing 0.5 mM ascorbate (10 ml/kg) or with 1 mg/kg of apomorphine either diluted from the pumps or dissolved fresh from the intact powdered stock drug (Merck, Sharp, and Dohme)[2] All animals were killed 30 min later by microwave irradiation focused on the head for 1.25 sec (Thermatron-Thermex Metabostat; 2.0 kW, 2450 MHz; Stoelting Co., Chicago, IL). The metabolites and dopamine in the caudate-putamen were measured by a GC-MS procedure using single ion monitoring (28). Compared with values from animals injected with vehicle, equivalent decreases in striatal concentrations of 3-MT to 20% (pump apomorphine) and 15% (powder apomorphine) and HVA to 66% (pump) and 65% (powder) of control levels were obtained ($p < 0.01$, Dunnett's t test). DOPAC was also decreased by either source of apomorphine ($p < 0.01$) but by 20% more ($p < 0.05$, Neuman-Keuls analysis) by stock powder-derived (to 38% of control) than by pump-derived (to 58% of control) apomorphine.

Drug stability and release studies—CGS 15855A. The *in vitro* release of CGS 15855A from the pumps was evaluated by dissolving 851 or 8509 μg of 15855A in 0.9% saline and loading the solutions into pumps (n = 3 per concentration). Each pump was immersed in 25 ml of 0.9% saline in capped 50-ml centrifuge tubes maintained for 14 days in a shaker water bath at 37°. The pumps were placed in new tubes containing fresh saline twice a day for 14 days. The release and chemical stability of CGS 15855A at 37° were determined by removing triplicate aliquots daily from the centrifuge tubes. After 14 days, the pumps were

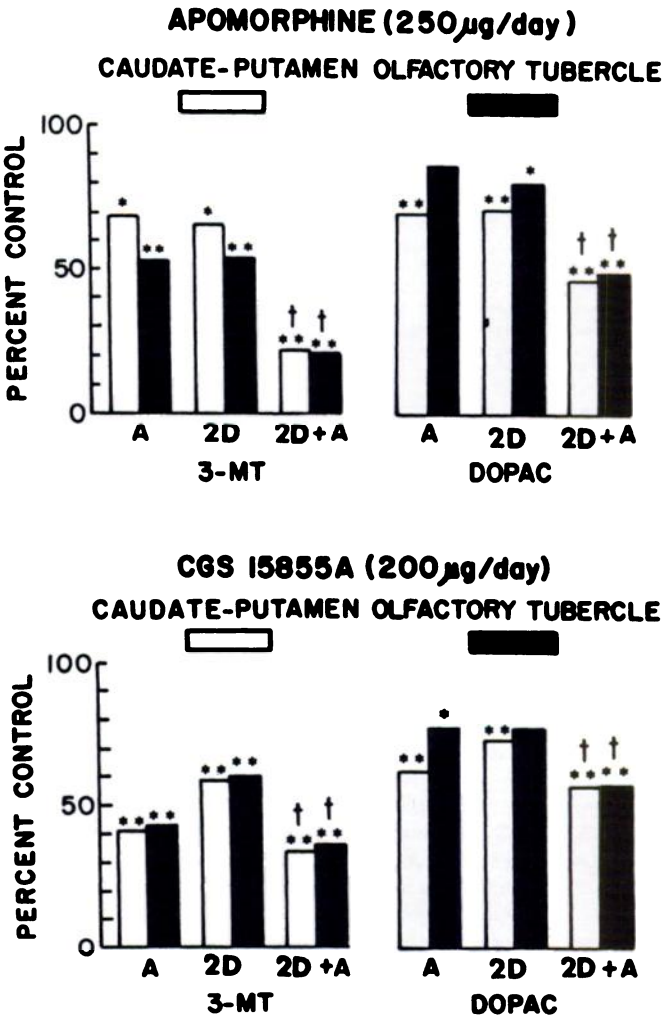

Fig. 1. Effect of 2-day apomorphine (*top panel*) and CGS 15855A (*bottom panel*) on dopamine release (3-MT concentrations) and metabolism (DOPAC concentrations) in the caudate-putamen (n = 7 per group) and olfactory tubercle (n = 5–7 per group). *Abcissa*: *A*, IP injection of 2 mg/kg apomorphine or 1 mg/kg CGS 15855A with sacrifice 30 min thereafter; *2D*, 2-day treatment with apomorphine (250 μg/day) or CGS 15855A (200 μg/day); *2D + A*, 2-day treatment followed by acute treatment with the same drug given for 2 days. As with Figs. 2 and 3, the standard error for each group was less than ±10%. *$p < 0.05$; **$p < 0.01$ versus animals treated with vehicle for 2 days and acutely, Dunnett's test on the corresponding concentration values; †, $p < 0.05$ versus *2D* group, Neuman-Keuls analysis. Vehicle values (pmol/mg of protein) were as follows: caudate-putamen—3-MT, 3.2 ± 0.4; DOPAC, 103 ± 3; HVA, 66 ± 3; and dopamine, 272 ± 13; olfactory tubercle—3-MT, 0.8 ± 0.1; DOPAC, 93 ± 8; HVA, 31 ± 2; and dopamine, 338 ± 34. Data not shown: dopamine values were unchanged from vehicle by any treatment except for a 32% increase in the caudate-putamen in animals treated for 2 days and 30 min with apomorphine. HVA changes and significance levels were very similar to those shown for DOPAC.

[2] The 1 mg/kg SC dose of apomorphine decreases DOPAC, HVA, and 3-MT in the mouse striatum by approximately half of the maximal suppression obtainable with higher apomorphine doses (22).

TABLE 1

Effects of 2-day treatment with CGS 15855A on dopamine release and metabolism in caudate-putamen and olfactory tubercle

Rats (180–220 g) were implanted with Alzet pumps, which delivered various doses of CGS 15855A daily. Vehicle pumps were loaded with 1% ascorbic acid. Two days later animals were challenged with vehicle IP and sacrificed 30 min later. Dopamine concentrations of the vehicle group were 272 ± 13 (mean ± SE) in the caudate-putamen and 338 ± 34 in the olfactory tubercle and were unchanged by any treatment.

Two-day treatment	Dose	Concentration					
		Caudate-putamen[a]			Olfactory tubercle[b]		
		3-MT	DOPAC	HVA	3-MT	DOPAC	HVA
	μg/day	pmol/mg of protein					
Vehicle	0	3.2 ± 0.4	103 ± 3	66 ± 3	0.76 ± 0.10	93 ± 8	31 ± 2
CGS 15855A	50	1.7 ± 0.2[c]	73 ± 3[c]	47 ± 2[c]	0.53 ± 0.10	74 ± 3	24 ± 1[d]
CGS 15855A	100	1.8 ± 0.2[c]	77 ± 4[c]	49 ± 2[c]	0.54 ± 0.12	63 ± 7[c]	19 ± 2[c]
CGS 15855A	200	1.9 ± 0.2[c]	75 ± 5[c]	49 ± 3[c]	0.46 ± 0.06[d]	77 ± 6	25 ± 3

[a] $n = 7$ per group.
[b] $n = 5$–7 per group.
[c] $p < 0.01$ versus vehicle group, Dunnett's t test.
[d] $p < 0.05$ versus vehicle group, Dunnett's t test.

TABLE 2

Plasma and brain levels of CGS 15855A administered for 14 days

The concentration of CGS 15855A were measured in the plasma and brain (whole brain minus caudate-putamen and olfactory tubercle) of rats (180–200 g; $n = 7$ per group) that had been implanted for 14 days with Alzet pumps containing either the vehicle (0 group) or one of three concentrations of CGS 15855A. On the fourteenth day, the animals were challenged IP with CGS 15855A (1.0 mg/kg) and killed 30 min thereafter. Values are mean ± standard error of seven animals per group.

Daily dose	CGS 15855A concentration	
	Plasma	Brain
μg	ng/ml	pg/mg of dry weight
0	ND[a]	ND
40	1.3 ± 0.26	21 ± 4.5
100	1.8 ± 0.45	31 ± 14
400	5.2 ± 0.94[b]	103 ± 12[b]

[a] ND, presence of drug was not detected.
[b] $p < 0.01$ versus 40 μg/day group, Dunnett's multiple t test.

flushed with two volumes of saline, and the released and residual drug was measured by HPLC using a 15-cm c-18 Novapak column (Waters Chromatography Div., Milford, MA) and a mobile phase consisting of methanol/phosphate buffer (7:3). The buffer contained 0.02 M potassium monophosphate adjusted to pH 7.0 with phosphoric acid. This mobile phase gave a retention time of 2.7 min at a flow rate of 1 ml/min. CGS 15855A was detected with a UV detector set at 280 nm (Spectroflow model 783; Kratos, Ramsey, NJ). The injection volume was 20 μl. The HPLC was a Waters WISP model 710 with a Waters model 530 programmable solvent pump.

Sustained drug administration studies. On the afternoon of the second or fourteenth day, each vehicle- or drug-treated rat was injected IP either with the 0.5 mM ascorbic acid, 0.9% sterile saline vehicle (1 ml/kg) or with the vehicle containing apomorphine (2 mg/kg, SC) or CGS 15855A (1 mg/kg). Each rat was killed 30 min later by microwave irradiation focused on the head as described for mice but for 2.2 sec at 3.5 kW. The decreases of DOPAC, HVA, and 3-MT concentrations in the caudate-putamen and olfactory tubercle were measured by GC-MS (28).

Plasma samples were obtained from blood collected from the trunks of animals administered CGS 15855A at daily doses of 50, 100, or 200 μg for 2 days and 40, 100, or 400 μg for 14 days. CGS 15855A amounts in the plasma or brain, minus caudate-putamen, were measured by GC-MS and CGS 15855A amounts within the pumps were determined by HPLC.

The pumps were removed for a determination of the amount of CGS 15855A that remained in each pump. The intended delivery rates of 40, 100, and 400 μg/day were obtained by loading the Alzet model 2 ML2 pumps with 851, 2127, and 8509 μg of CGS 15855A. Drug output per day was calculated on the basis of drug recovery and the residual drug in the pumps at 14 days. The *in vitro* and *in vivo* drug outputs for 14 days were, respectively, for the lowest dose (mean ± SD) 40 ± 2 and 44 ± 2 μg/day, 110 ± 10 μg/day for the intermediate dose, and 423 ± 3 and 408 ± 20 μg/day, for the highest dose. These measures, as well as visual inspections of the HPLC chromatograms, indicated that no degradation of the drug had occurred and that the drug was administered at the desired rates.

Results

Two-Day Drug Administration

Steady state plasma levels of CGS 15855A after SC implantation for 2 days. The average plasma concentrations

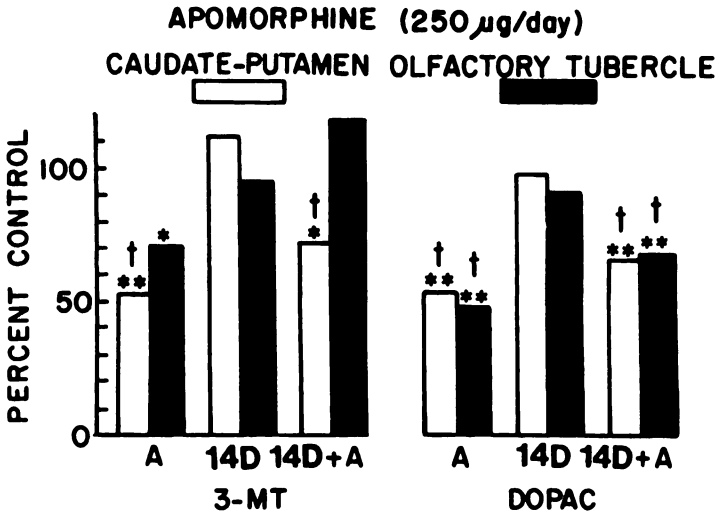

Fig. 2. Effect of apomorphine (250 μg/day for 14 days; $n = 5$–7 per group) on dopamine release and metabolism in the caudate-putamen and olfactory tubercle. *Abscissa* abbreviations are the same as in Fig. 1 except that *14D* = 14 day administration via Alzet pumps. *$p < 0.05$, **$p < 0.01$ versus animals treated with vehicle for 14 days and acutely; †$p < 0.05$ versus 14-day group. Vehicle values (pmol/mg of protein) were as follows: caudate-putamen—3-MT, 2.4 ± 0.2; DOPAC, 83 ± 6; HVA, 87 ± 7; and dopamine, 391 ± 20; olfactory tubercle—3-MT, 2.1 ± 0.1; DOPAC, 100 ± 8; HVA, 60 ± 5; and dopamine, 528 ± 32. Dopamine values were unchanged from vehicle by any treatment, except for an increase ($p < 0.01$) in the caudate-putamen of the 14D + A group. HVA values changed in a nearly identical manner to DOPAC in each tissue.

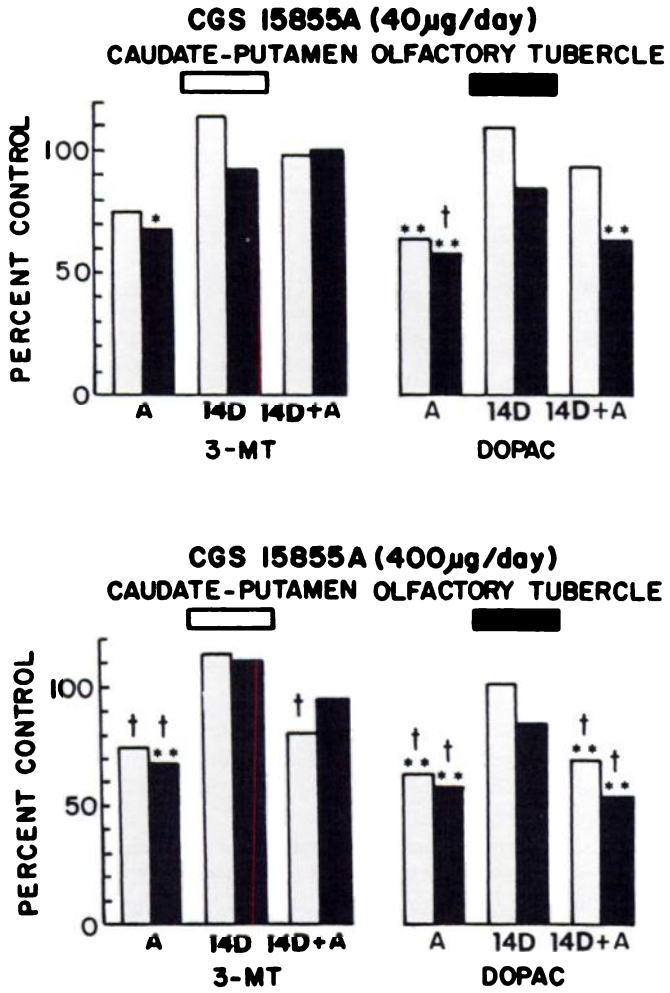

Fig. 3. Effect of 14-day administration of CGS 15855A at 40 μg/day (*top panel*; $n = 5$–7 per group) or 400 μg/day (*bottom panel*; $n = 7$ per group) on dopamine release and metabolism in the caudate-putamen and olfactory tubercle. Abcissa abbreviations are the same as in Fig. 1 except that 14D = 14-day administration. $^{*}p < 0.05$, $^{**}p < 0.01$ versus animals treated with vehicle for 14 days and acutely, $\dagger p < 0.05$ versus 14-day group. Vehicle values (pmol/mg of protein) are the same as in Fig. 2. Dopamine values were unchanged from vehicle by any treatment, except for increases in the 14D (38%) and 14D + A (32%) groups (50 μg/day) in the caudate-putamen. HVA values changed in a nearly identical manner to DOPAC in the caudate-putamen (400 μg/day) but did not change in any other condition.

of CGS 15855A increased linearly as a function of the intended daily drug dose after 2 days of CGS 15855A delivery. The mean plasma concentrations for the 100 and 200 μg/day delivery rates were 6.1 ± 1.5 and 12.9 ± 9.7 ng/ml plasma (mean ± SD; $n = 7$ per group). These values did not differ from each other but exceeded the mean of 3.9 ± 1.4 ng/ml plasma for the 50-μg/day group ($p < 0.01$, Dunnett's t test).

Neurochemical effects of two-day autoreceptor agonist administration. Basal concentrations of dopamine, DOPAC, HVA, and 3-MT in the caudate-putamen and olfactory tubercle of mice were within the range of reported values (22, 23). Concentrations of each metabolite were decreased in the caudate-putamen and olfactory tubercle 30 min after the acute 2 mg/kg IP injection of apomorphine and after 2 days of apomorphine administration (Fig. 1, *top panel*). Acute apomorphine injections in animals treated for 2 days with apomorphine produced a suppression of HVA (data not shown) and 3-MT and DOPAC (Fig. 1, *top panel*) in each tissue that consistently exceeded the suppressions produced by sustained administration of apomorphine alone.

DOPAC, HVA, and 3-MT were decreased equally in the caudate-putamen after 2-day administration of 50, 100, and 200 μg/day of CGS 15855A (Table 1). The concentrations of 3-MT, DOPAC, and/or HVA were also decreased in the olfactory tubercle after each dose of CGS 15855A (Table 1). DOPAC and HVA were consistently suppressed in the caudate-putamen and olfactory tubercle 30 min after vehicle injections in animals receiving CGS 15855A for 2 days (Fig. 1, *bottom panel*).

Intraperitoneal injections of 1.0 mg/kg of CGS 15855A into animals treated for 2 days with 200 μg/day of CGS 15855A consistently suppressed 3-MT and DOPAC beyond the suppression produced by sustained drug administration alone (Fig. 1, *bottom panel*).

Two-Week Drug Administration

Steady state plasma and brain levels of CGS 15855A after SC implantation for 14 days. The average plasma and brain concentrations of CGS 15855A increased linearly with the intended daily drug dose after 14 days of administration (Table 2). The plasma concentrations of CGS 15855A were less than those reported for 2-day administration of comparable doses.

Neurochemical effects of 14 day autoreceptor agonist administration. As observed in animals administered vehicle for 2 days, those receiving vehicle for 2 weeks showed a marked suppression of DOPAC and 3-MT in the caudate-putamen and olfactory tubercle 30 min after apomorphine (Fig. 2) or CGS 15855A (Fig. 3). In marked contrast to 2-day administration, however, 14 days of apomorphine (Fig. 2) or CGS 15855A (Fig. 3; Table 3) did not decrease HVA (data not shown), 3-MT, or DOPAC in either tissue.

Dopamine concentrations of the vehicle group were 391 ± 20 (mean ± SE) in the caudate-putamen and 528 ± 32 in the olfactory tubercle and were increased by 38 and 34% ($p < 0.05$, Dunnett's t test) in the caudate-putamen of animals receiving 40 or 100 μg of CGS 15855A per day, respectively. Changes only in dopamine (20–57% increases) were again observed in a subsequent 14-day study using identical treatments (data not shown).

The combination of an acute injection and 14-day delivery of apomorphine (Fig. 2) or CGS 15855A (Fig. 3) decreased HVA in only about half of the metabolite measurement groups (data not shown), decreased dopamine release (3-MT) in only one of the six groups of animals treated with either agonists, and decreased dopamine metabolism (DOPAC) in five of six of the agonist-treated groups. Compared with the 40-μg/day CGS 15855A treatment, the 400-μg/day treatment was associated with a greater ability of acute CGS 15855A injections to lower DOPAC below levels attained with chronic treatment only (Fig. 3).

Discussion

Reports of tolerance within 1 week to chronic dopamine agonist treatments have appeared in both the experimental (12, 17, 19, 27, 29–31) and clinical (15, 16) literature. The present studies determined whether constant delivery of apomorphine or the recently introduced selective dopamine autoreceptor agonist CGS 15855A (21, 22) could obviate tolerance. In addi-

TABLE 3

Effects of 14-day treatment with CGS 15855A on dopamine release and metabolism in caudate-putamen and olfactory tubercle

Rats were implanted with Alzet pumps as described in Table 2. Animals were challenged fourteen days later with vehicle IP and killed 30 min later. Dopamine concentrations of the vehicle group were 391 ± 20 (mean ± SE) in the caudate-putamen and 528 ± 32 in the olfactory tubercle. Increases of 34 and 38% ($p < 0.05$) in striatal but not olfactory tubercle dopamine concentrations occurred in the 40 and 100 μg/day groups, respectively. n = 6–7 per group.

Chronic treatment	Daily dose	Concentration					
		Caudate-putamen			Olfactory tubercle		
		3-MT	DOPAC	HVA	3-MT	DOPAC	HVA
	μg	pmol/mg of protein					
Vehicle	0	2.4 ± 0.2	83 ± 6	87 ± 7	2.1 ± 0.1	100 ± 8	60 ± 5
CGS 15855A	40	2.9 ± 0.2	100 ± 6	97 ± 4	2.2 ± 0.3	95 ± 6	64 ± 5
CGS 15855A	100	2.8 ± 0.2	100 ± 5	102 ± 5	1.9 ± 0.3	85 ± 7	56 ± 5
CGS 15855A	400	2.8 ± 0.2	85 ± 9	93 ± 7	2.3 ± 0.1	86 ± 2	60 ± 2

tion, this study measured for the first time whether the release-suppressing properties of dopamine agonists would persist with their sustained administration.

Two-day autoreceptor agonist administration. The absence of tolerance to either agonist at 2 days was shown by the considerable drug potency for the suppression of striatal and limbic dopamine neurochemistry. Continuous infusions of at most 10 μg/hr of apomorphine and 2 μg/hr of CGS 15855A were sufficient at 2 days to greatly suppress dopamine metabolism and release. By comparison, a 1 mg/kg IP dose of CGS 15855A only half-maximally suppresses striatal dopamine release and metabolism and does so for only up to 90 min (22). Either the SC route of administration, the constant delivery of agonist, or both account for the greater potency of CGS 15855A given by the Alzet pump, compared with the IP route. Because only a 4-fold greater SC versus IP potency of apomorphine (32) or CGS 15855A[3] is obtained, the continuous form of delivery via the minipump is likely to account for part of the approximately 100-fold greater potency of pump-delivered versus bolus-injected CGS 15855A in suppressing dopamine metabolism and release.

The lack of tolerance at the 2-day time was also indicated by the consistent ability of acute agonist treatments to suppress metabolite levels below those obtained with the drug delivered for 2 days.

Fourteen-day autoreceptor agonist administration. Tolerance to the dopamine release- and metabolism-suppressing properties of dopamine autoreceptor agonists developed between 2 and 14 days of sustained delivery. In marked contrast to the 2-day administration of apomorphine or CGS 15855A, 14-day administration of either drug did not suppress any metabolite in either mesolimbic or striatal regions. Even a daily 400-μg dose of CGS 15855A, which exceeded by 8-fold a maximally effective 50-μg/day dose at 2 days, did not suppress any metabolite at 2 weeks. Also, compared with treatment for 2 days, 14-day treatment markedly attenuated the ability of an acute drug challenge to suppress levels of any metabolite. Challenge for 30 min with the agonist administered for 2 weeks failed in 6 of 12 DOPAC and 3-MT measurement groups to decrease metabolite levels, even though the same drug was also being delivered to these animals via the Alzet pumps. These results are consistent with studies showing decreased autoreceptor responses after chronic administration of apomorphine, 3-PPP, EMD-23,448, bromocriptine, and *d*-amphetamine (17–19, 27, 29–31, 33, 34). Thus, tolerance to the dopamine release- and metabolism-suppressing properties of dopamine autoreceptor agonists developed between 2 and 14 days of sustained delivery. Because plasma levels of CGS 15855A were less at 14 than at 2 days of comparable dose administration, it is possible that an enhanced rate of CGS 15855A degradation may have developed during this period. This would not fully explain the inability of the highest dose to lower metabolites at 14 days, however, because plasma levels *exceeded* those after 2 days of the 50 μg/day dose, which maximally suppressed all metabolites.

A similar time course has been obtained in humans for tolerance for apomorphine treatment of schizophrenia (15, 16). Interestingly, unlike the amygdala, olfactory tubercle, and the more commonly studied striatal regions (32, 34, 35), neocortical areas including the frontal, cingulate, and suprarhinal cortices do not show tolerance to chronic treatments with *d*-amphetamine (30) or D_2 receptor antagonists (36–38). Thus, the loss of antipsychotic efficacy after several days of treatment with apomorphine or *N*-(*n*-propyl)norapormorphine (15, 16) indicates that the transient antipsychotic action of these drugs may result from a suppression of limbic or striatal and not cortical dopaminergic activity.

Dopamine synthesis and release, as measured by DOPAC and 3-MT, respectively, returned to normal after 2 weeks of administration of either agonist. However, dopamine levels of the striatum remained elevated by 34–38% after 14 days of CGS 15855A (Table 3) and by 20–57% (data not shown) in the striata of animals for which plasma and brain CGS 15855A levels were reported in Table 2. Thus, although the majority of evidence indicates that mesolimbic and mesostriatal dopamine neurons show tolerance to chronic administration of dopamine agonists, the persistence of elevated dopamine levels after chronic and chronic plus acute CGS 15855A, and some ability of chronic plus acute CGS 15855A to lower DOPAC, indicates that tolerance does not extend to all striatal actions of CGS 15855A.

The continued release, metabolism, and synthesis of dopamine in neurons that are tolerant to autoreceptor-selective and nonselective dopamine agonists indicate potential clinical uses for these drugs. For example, sustained administration of CGS 15855A will diminish the responsiveness of limbic as well as striatal dopamine autoreceptors and may therefore ameliorate positive symptoms of schizophrenia that may result from sensitive autoreceptors (11). The higher doses of CGS 15855A, which after chronic administration did not suppress ongoing dopamine release, might be useful in the treatment of Parkinson's disease by allowing normal amounts of striatal dopamine

[3] C. A. Anthony and W. C. Boyar, unpublished observations.

release to occur concomitantly with stimulation of supersensitive postsynaptic D_2 receptors.

Tolerance in the present studies also developed after sustained delivery of apomorphine, a relatively nonselective but potent D_2 dopamine autoreceptor agonist (21, 34). Presumably, tolerance to CGS 15855A and apomorphine may result from a decreased concentration of the dopamine D_2 receptors or a subsensitivity of adenylate cyclase, as observed after chronic administration of bromocriptine (19), apomorphine (39), or *d*-amphetamine (30). Together with the present findings, the data obtained thus far indicate that neither the degree of autoreceptor selectivity, route of drug administration, nor bolus versus controlled form of delivery will prevent tolerance to probably the most important presynaptic component of autoreceptor control, the modulation of dopamine release.

Acknowledgments

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