Pharmacology Biochemistry and Behavior, Vol. 43, pp. 929–937, 1992
Printed in the U.S.A.

0091-3057/92 $5.00 + .00

RAPID COMMUNICATION

Distinguishing Effects of Cocaine IV and SC on Mesoaccumbens Dopamine and Serotonin Release With Chloral Hydrate Anesthesia

PATRICIA A. BRODERICK[1]

Department of Pharmacology, The City University of New York Medical School and Departments of Biology and Psychology, CUNY Graduate School, New York, NY 10031

Received 24 June 1992

BRODERICK, P. A. *Distinguishing effects of cocaine IV and SC on mesoaccumbens dopamine and serotonin release with chloral hydrate anesthesia.* PHARMACOL BIOCHEM BEHAV **43**(3) 929–937, 1992.—The effect of IV cocaine (0.5 and 1.0 mg/kg) was studied on synaptic concentrations of dopamine (DA) and serotonin [5-hydroxytryptamine (5-HT)] in the mesoaccumbens nerve terminal, the nucleus accumbens (NAcc), in chloral hydrate-anesthetized, male Sprague-Dawley rats (*Rattus norvegicus*) with in vivo electrochemistry (voltammetry). In further in vivo voltammetric studies, the effects of SC cocaine on synaptic concentrations of DA and 5-HT were studied in the chloral hydrate-anesthetized paradigm in two neuroanatomic substrates, NAcc and mesoaccumbens somatodendrites, the ventral tegmental area (VTA-A_{10}), in a dose-response fashion (10, 20, and 40 mg/kg) in six separate studies. Moreover, in two additional in vivo voltammetric studies, again using the chloral hydrate-anesthetized paradigm, the impulse flow blocker, γ-butyrolactone (γ-BL) (750 mg/kg, IP), was studied alone and in combination with SC cocaine (20 mg/kg) to determine whether or not cocaine can act by presynaptic releasing mechanisms for DA and 5-HT. The results show that IV cocaine concurrently and significantly increased DA and 5-HT release in the NAcc ($p < 0.001$, $p < 0.0005$, respectively) at both doses tested. Moreover, IV cocaine effects on DA and 5-HT release were significantly and positively correlated ($p < 0.01$). On the other hand, SC cocaine concurrently and significantly decreased DA and 5-HT release in NAcc ($p < 0.0001$) and VTA ($p < 0.0001$) at each separate dose tested. SC cocaine effects on DA and 5-HT release were significantly and positively correlated across dose and neuroanatomic substrate ($p < 0.01$). Furthermore, the γ-BL studies indicate that cocaine's action includes a presynaptic release mechanism for the biogenic amines. Summarily, the data show that a consideration of the route of cocaine administration is crucial in determining the underlying neurochemical basis for cocaine.

Cocaine Dopamine Serotonin Ventrolateral nucleus accumbens In vivo electrochemistry (voltammetry) Chloral hydrate anesthesia Ventral tegmental area

THERE is a general consensus from clinical and preclinical studies that cocaine has specific dopaminergic (DAergic) influences associated with its craving or craving-like syndrome (15,20,25,30,42–44,46). Predominantly, the evidence shows that the behavioral types of cocaine reinforcement involve an operative mesoaccumbens DAergic hyperfunction in A_{10} nerve terminals [nucleus accumbens (NAcc)] (7,11,24,27,40) and in A_{10} somatodendrites [ventral tegmental area (VTA)] (9). Of necessity, though, the correlation between cocaine-induced enhancement of dopamine (DA) overflow and its associated behavior was demonstrated in the freely moving animal paradigm. In this paradigm, adequate recovery from surgery precludes anesthetic consequence. On the other hand, studies of the electrophysiological effects of cocaine on impulse frequency rates (cell firing) of DA neurons in the VTA have been examined in animals anesthetized with chloral hydrate (16). Therefore, because pre- and postsynaptic release mechanisms are contingent upon somatodendritic impulse frequency rates, nuances in DA cell responsiveness due to either or both general and local anesthesia deserve consideration.

Recent reports have shown that chloral hydrate, a general anesthetic, changes a) the basal activity, b) the pharmacologi-

[1]Requests for reprints should be addressed to Dr. Patricia A. Broderick, Department of Pharmacology, Room J-910, The City University of New York Medical School, and Departments of Biology and Psychology, CUNY Graduate School, Convent Avenue and West 138th Street, New York, NY 10031.

cal responsiveness, and c) the spontaneous activity (burst firing) of mesolimbic mesoaccumbens DA neurons; suppression of burst firing occurs in a continuum with effects of general anesthetics > local anesthetics > no anesthetic (18,28). Given this caveat, we assessed the effects of IV cocaine on synaptic concentrations of DA in the NAcc and VTA in the chloral hydrate-anesthetized paradigm. The IV route of administration was chosen for direct comparison to electrophysiological studies. Moreover, further studies were done to assess the effect of cocaine when it is administered SC because no neurochemical studies of this kind have been addressed, despite the early data distinguishing the pharmacokinetic effects of IV and SC cocaine (36).

The monoamine-containing neurons for DA (the A class) and serotonin [5-hydroxytryptamine (5-HT)] (the B class) have been described by Dahlstrom and Fuxe (14). At first thought to be distinct and separate neurotransmitters in distinct and separate neuronal fiber pathways, a rethinking, commingling these monoamines, has emerged appendant to the demonstration of a wide occurrence of 5-HT positive nerve terminals throughout the CNS (47). A sequence of events occurred in the analytic study of the 5-HT organization in brain that initially demonstrated a prominent transtegmental 5-HT fiber pathway in the A_{10} region that begins in the median and dorsal mesencephalic raphe nuclei and curves ventrally across the VTA to communicate in the medial forebrain bundle (MFB) (38,48). Subsequently, 5-HT axons project from the MFB to NAcc (1). A more recent study shows that 5-HT axons directly innervate DA neurons in the VTA (23).

Interestingly, empirical evidence for a 5-HTergic mediation or intermediation with mesoaccumbens DA in cocaine reinforcement behavior has also been gleaned using the freely moving rat animal model (7–9,32). Yet, as is the case with the scientific literature regarding DAergic impulse frequency rates in the VTA, the 5-HT electrophysiological data were derived from studies of cocaine's effects on impulse frequency rates in the dorsal raphe (DR) in the chloral hydrate-anesthetized paradigm (13). Accordingly, we study here the effects of cocaine on 5-HT release in the NAcc and VTA sequentially and on line with DA release in the chloral hydrate-anesthetized paradigm.

Release mechanisms are evidenced by an assessment of the electroactive species for DA and 5-HT using the impulse flow inhibitor, γ-butyrolactone (γ-BL) (49). Release mechanisms are known to be dependent upon the frequency of impulse flow whereas uptake processes are said to be time dependent (34).

METHOD

Surgical Procedures, Chemicals, and Protocols

Male Sprague-Dawley rats were purchased virus free from Charles River Laboratories (Kingston, NY). The specific viruses tested include: Sendai Virus, Kilham Rat Virus, Reo Virus Type 3, Sialodacryoadenitis Virus, Rat Corona Virus, Toolan's H1 Virus, Micro Plasma Pulmonis Virus, Lymphocytic Choriomeningitis Virus, Hantaan Virus, and Encephalitozoon Cuniculi Virus. Rats weighed 261–372 g at the time of the study. Animals were group housed and fed Purina Rat Chow and water ad lib; a 12 L:12 D cycle was maintained.

Rats were anesthetized for surgery with an IP injection of chloral hydrate (450 mg/kg). An additional injection of chloral hydrate (0.1 cc) was administered every 40 min to maintain adequate depth of anesthesia; leg flexion and corneal and pinnal responses were assessed. The indicator (working) microelectrode was slowly inserted (0.5 mm in 1 min at 5-min intervals) into ventrolateral NAcc and into VTA in separate groups of animals using the stereotaxic coordinates of Pelligrino and Cushman (39). The stereotaxic coordinates were: NAcc: from bregma A = 2.6, ML = +2.5, and DV = −7.3; VTA: from bregma P = 2.8, ML = +0.9, and DV = −8.6. An Ag/AgCl reference microelectrode and stainless steel auxiliary microelectrode combination was placed in contact with dura. Each microelectrode of the three-microelectrode assembly was constructed in this laboratory. Body temperature was continuously monitored with a rectal probe attached to a thermometer (Fisher Scientific, Fadem, NJ) and was continuously maintained at 37 ± 0.5°C with an Aquamatic K module heating pad (American Hospital Supply, Edison, NJ). This was done a) to prevent electrochemical peak potential (E_{ap}) shifts because E_{ap} is temperature dependent and b) to prevent anesthetic-induced hypothermia. Each animal was treated with a great deal of care throughout the studies.

Cocaine HCl (Sigma Chemical Co., St. Louis, MO) was dissolved in deionized, organic free water (resistance = 5–10 MΩ). The solution was made fresh on each day of study. Cocaine was administered IV after each in vivo electrochemical signal for DA and 5-HT in the NAcc was uniform within SE ± 5%, for at least 40 min. Cocaine effects on the separate electroactive species for DA and 5-HT were studied concurrently for 1 h at a dose of 0.5 mg/kg and at a subsequent dose of 1.0 mg/kg for 1 h. Cocaine was administered IV through cannulation of the femoral vein. The femoral vein was cannulated with 0.965 mm o.d. tubing (PE-50, Clay Adams, Parsippany, NJ). Femoral vein cannulation was completed within 15 min. The time for the entire surgical procedure and study was approximately 5.5 h.

Moreover, cocaine was administered SC to six different groups of animals: Three groups had working microelectrodes placed in the ventrolateral NAcc; three groups had working microelectrodes placed in the VTA. A dose–response study was done on the separate effects of 10, 20, and 40 mg/kg SC cocaine on synaptic concentrations of DA and 5-HT in the synaptic environment of either the NAcc or VTA. Cocaine was administered SC after each in vivo electrochemical signal for DA and for 5-HT was uniform within SE ± 5% for at least 40 min. SC cocaine effects were studied for 3 h after cocaine administration because of its reported longer half-life ($t_{1/2}$) in brain (36).

Studies were also done to address a possible presynaptic mechanism of action of release for cocaine by using the known DA neuronal impulse blocker, γ-BL. The effect of γ-BL is equivalent to axotomy (49). γ-BL [750 mg/kg, IP (corrected for density at 1.12 g/cc)] was purchased in liquid form (Sigma, St. Louis, MO). It was administered to two different groups of male Sprague-Dawley rats. In one group, after the DA and 5-HT electrochemical signals were uniform within SE ± 5% for at least 40 min, the effect of γ-BL on the electroactive species for DA and 5-HT was studied for 40–60 min. In the other group, after the same stabile environment for basal synaptic concentrations of DA and 5-HT was evident and after one third of the DAergic impulse flow was blocked by γ-BL, cocaine (20 mg/kg, SC) was administered. The effects of SC cocaine on synaptic concentrations of DA and 5-HT in the NAcc was studied for 1 h after neuronal impulse inhibition by γ-BL. The γ-BL impulse flow inhibition studies were done in the SC cocaine-anesthetized paradigm in NAcc for direct

comparison with previous γ-BL–cocaine studies completed in the freely moving animal paradigm (8).

In Vivo Electrochemical (Voltammetric) Biotechnology

The methods for the formulation of in vivo electrochemical working microelectrodes including the conditioning or preconcentration steps and the specifications for the synthesis of the stearic acid carbon paste are published by this laboratory (5). A review of the historic and technical aspects of the field of in vivo electrochemistry is referenced (6). Electrocatalytic interactions between DA and ascorbic acid (AA) have been reported with a stearic acid macroelectrode in vitro (21), but more recent reports show that these interactions are insignificant in neuronal tissue in vivo when a stearic acid microelectrode is used (2).

In the present studies, stearate microelectrodes (size = 200 μm diameter; 500 μm length) were used in combination with semidifferential (semiderivative) voltammetry. Dopamine and 5-HT were detected within 10–15 and 10–13 s, respectively, and sequentially in two separate waveforms on the in vitro and in vivo voltammogram in both the NAcc and VTA. DA was the first signal and 5-HT the second to be detected in the time course of the applied potential (E_{app}). E_{ap} for DA is $+0.140 \pm 0.015$ V. Even at high concentrations, the anionic metabolite of DA, 3,4-dihydroxyphenylacetic acid (DOPAC), was not detected. At high concentrations, however, ascorbic acid (AA), the cofactor in the DA biosynthetic pathway, was detectable but at a different E_{ap} than that for DA or 5-HT. The neurotransmitter 5-HT was detected within a 10–13 s time period at an E_{ap} of $+0.290 \pm 0.015$ V. Neither the metabolite of 5-HT, that is, 5-hydroxyindoleacetic acid (5-HIAA), nor uric acid (UA), which is a constituent of brain with similar electroactive E_{ap} properties to those of 5-HT, was part of the 5-HT signal. In vivo electrochemical parameters such as E_{ap} for DA and 5-HT are the same in the VTA as in NAcc, given that conditions of temperature, pH, resistance, capacitance, time constants, sensitivities, and preconcentration procedures are not changed. Each voltammogram is completed within 60 s in vivo. Initial potential (E_{app}) was -0.200 V; the end E_{app} was $+0.400$ V; the scan rate was 10 mV s^{-1}. Potentials were applied to the working microelectrode with respect to an Ag/AgCl microelectrode (with 0.16 M saline) by a CV37 detector potentiostat (BAS, West Lafayette, IN). The CV37 was electrically connected to a Minigard Surge Suppressor (Jefferson Electric, Magnetek, NY), which was then connected to an isolated electrical ground. There was a 6-min interval between the completion of one voltammogram and the 2-min cell activation for the next voltammogram. Non-Faradaic charging current (C_{dl}) was eliminated in the first 25 s of each scan. Coulombic efficiency for the detection of 5-HT was two- to threefold greater than that for DA as shown by previous in vitro studies (4,5).

Working microelectrodes were precalibrated in vitro in a fresh, deaerated saline (0.16 M) phosphate buffer (PO_4) solution, pH 7.4 (0.01 M) containing aliquots of nmol solutions of DA and 5-HT [DA (purity, 99%): Sigma; and 5-HT (purity, 99%): Aldrich, Milwaukee, WI]. The buffer was deareated with prepurified nitrogen gas (N_2) extra dry grade, 5 psi, (T.W. Smith Co., Brooklyn, NY). N_2 gas was perfused through the PO_4 buffer, which was regulated by a Variable Area Flowmeter (Purgemaster, Fisher Scientific Co., Fadem, NJ). The Variable Area Flowmeter was calibrated at 90–100 cc/min. Deaeration took place for 1 min before each cell activation period and subsequent voltammetric scan from E_i (Initial E_{app}) began. Working microelectrodes were also postcalibrated in vitro in a freshly deoxygenated PO_4 buffer solution pH 7.4 (0.01 M), made exactly as was the precalibration buffer, after each study was completed. Peak area of each DA and 5-HT signal was calculated by multiplying the peak height (mm) of each electrochemical signal by the width (mm) of each electrochemical signal at one half the peak height (mm). Detectable basal concentrations of DA and 5-HT, less than 5 and 1 nmol, respectively, are currently possible with this biotechnology.

Histological Procedure

Histological placements of working microelectrodes in the NAcc and VTA, in separate groups of animals, were confirmed by the potassium ferrocyanide blue dot method. The precise electrical specifications for deposition of the blue dot in the NAcc and VTA was 50 μA current in a 30-s time period. Virtually no damage to brain tissue occurred.

Statistics

Statistically significant differences between basal synaptic concentrations of DA and 5-HT vis-à-vis drug-affected synaptic concentrations of DA and 5-HT were determined by standard repeated-measures analysis of variance (ANOVA) (Statview, Brain Power Inc., Calabasas, CA). ANOVAs were followed by posthoc tests, Fisher's PLSD (least square differences), and Scheffe's *F*-test (Statview, Brain Power) to determine hourly statistically significant differences. Statistically significant differences were also calculated on the individual time course data points by 95% confidence limits (95% CL), setting the p value at $p < 0.05$. Changes in DA and 5-HT values after IV or SC cocaine treatment vis-à-vis untreated (same animal) controls are presented as % change to minimize normal between animal variations. Because the actual detection time for DA is 10–15 s, the % change in synaptic concentrations of DA at each data point, postcocaine, represents a 10–15 s current change (pA) from baseline, that is, current detected within a discrete synaptic environment of the NAcc or VTA at the microelectrode surface within a 10–15 s time period in the presence of cocaine vis-à-vis the absence of cocaine. The same principle of in vivo electrochemical detection applies for 5-HT. Because the actual detection time for 5-HT at each data point is 10–13 s, the % change in synaptic concentrations of 5-HT, at each data point postcocaine, represents a current measurement within the same discrete synaptic environment as that for DA, within the NAcc or VTA within a 10–13 s time period, in the presence of cocaine vis-à-vis the absence of cocaine. Control is represented as 100%. γ-BL data are also normalized. In vivo electrochemical signals for DA and 5-HT in the NAcc and VTA were studied for statistically significant correlative value by the Pearson Product Moment Coefficient of Correlation (r) (Statview, Brain Power); corresponding z values were derived from the table of z for values of r from 0.0–1.0.

RESULTS

Figure 1 shows time course effects for IV cocaine (0.5 and 1.0 mg/kg) on synaptic concentrations of DA and 5-HT in the ventrolateral NAcc in the chloral hydrate-anesthetized paradigm. IV cocaine significantly increased the electrochemical signal for DA over the 2-h two-dose period of testing

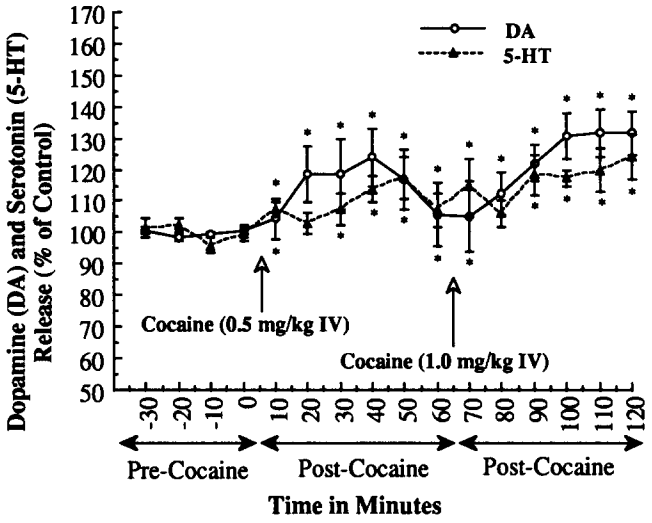


FIG. 1. Effect of IV cocaine (0.5 and 1.0 mg/kg) on synaptic concentrations of DA and 5-HT in the NAcc in the chloral hydrate-anesthetized paradigm (N = 5) (*p < 0.05, 95% CL) (cf. text for ANOVA statistics).

[ANOVA: $F(2, 10) = 15.04$, $p < 0.0010$, $N = 5$]. Posthoc analysis further showed that the statistically significant differences between basal synaptic concentrations of DA and cocaine-induced synaptic concentrations of DA occurred at each of the doses tested (0.5 mg/kg: Fisher's PLSD = 9.303, Scheffe's F = 6.264; 1.0 mg/kg: Fisher's PLSD = 9.303, Scheffe's F = 14.57). Cocaine significantly increased the in vivo electrochemical signal for 5-HT over the 2-h, two-dose period of testing [ANOVA: $F(2, 10) = 17.973$, $p < 0.0005$, $N = 5$]. Posthoc analysis further revealed that the statistically significant differences between endogenous synaptic concentrations of 5-HT and cocaine-induced synaptic concentrations of 5-HT took place at each of the doses tested (0.5 mg/kg: Fisher's PLSD = 6.239, Scheffe's F = 5.675; 1.0 mg/kg: Fisher's PLSD = 6.239, Scheffe's F = 17.877). The 5-HTergic responsiveness to cocaine very closely parallels that of DA. DA and 5-HT values are significantly and positively correlated [Pearson product: $r_{(a)} = 0.810$, $z = 1.1270$, $p < 0.01$]. At the completion of the 0.5-mg/kg dose effect, DA and 5-HT return to baseline to 106 and 107% above basal values, respectively. DA and 5-HT are coincident immediately after and 50 and 60 min after the lower dose of IV cocaine and at 30 min after the higher dose of IV cocaine. Both DA and 5-HT increase after IV cocaine in a dose-dependent fashion.

Figures 2a-c show the temporal effects of SC cocaine on synaptic concentrations of DA in the NAcc and VTA in the chloral hydrate-anesthetized paradigm. Figure 2(a) shows that SC cocaine (10 mg/kg) significantly decreased synaptic concentrations of DA in the NAcc and VTA [NAcc: ANOVA, $F(3, 15) = 94.093$, $p < 0.0001$, $N = 5$; VTA: ANOVA, $F(3, 15) = 39.837$, $p < 0.0001$, $N = 5$]. Posthoc analysis revealed that significant decreases in synaptic concentrations of DA occurred in each of the 3 h tested after SC cocaine administration, in both nerve terminals, NAcc and somatodendrites, VTA [A_{10}] (NAcc: Fisher's PLSD = 2.425, Scheffe's F = 34.752, 66.874, and 73.884 for h 1-3, respectively; VTA: Fisher's PLSD = 6.677, Scheffe's F = 13.858, 37.989, and 17.865

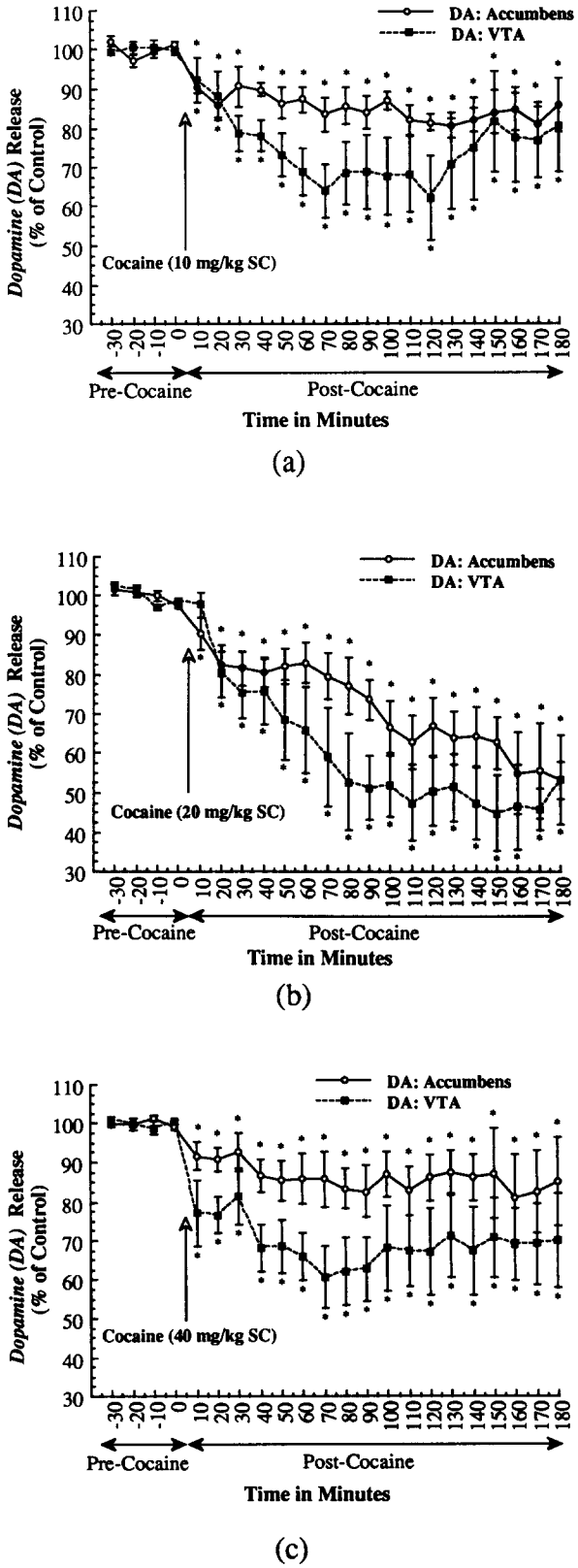


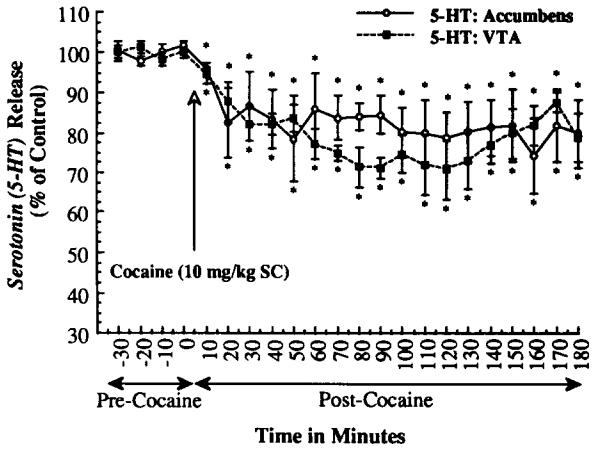
FIG. 2. Effect of SC cocaine on synaptic concentrations of DA in the NAcc and VTA in the chloral hydrate-anesthetized paradigm. (a) 10 mg/kg, N = 5. (b) 20 mg/kg, N = 5. (c) 40 mg/kg, N = 4-7. *p < 0.05, 95% CL (cf. text for ANOVA statistics).

for h 1-3, respectively]. The mesolimbic neuroanatomic substrate most affected by SC cocaine was the VTA [ANOVA, $F(1, 5) = 307.918, p < 0.0001, N = 5$; $F(1, 5) = 30.548, p < 0.0027, N = 5$, h 2 and 3, respectively]. DA in the NAcc and VTA was significantly and positively correlated [Pearson product: $r_{(a)} = 0.843, z = 1.2212, p < 0.01$].

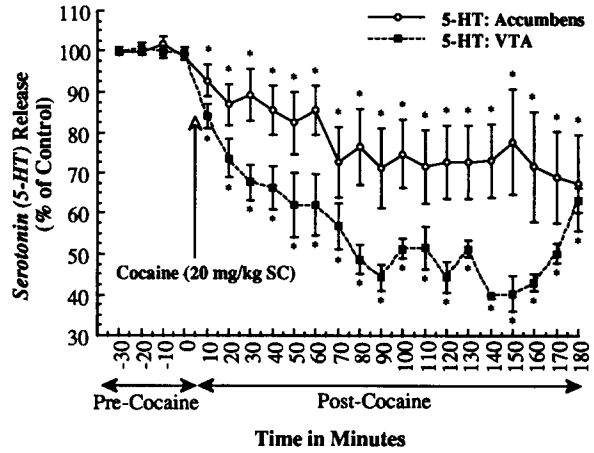
Figure 2(b) shows the temporal effects of SC cocaine (20 mg/kg). SC cocaine significantly decreased DA in both the NAcc and VTA [NAcc: ANOVA, $F(3, 15) = 184.651, p < 0.0001, N = 5$; VTA: ANOVA, $F(3, 15) = 126.324, p < 0.0001, N = 5$]. Posthoc analysis further revealed that the data from each of the 3-h postcocaine assays were statistically significant over control (basal) values (NAcc: Fisher's PLSD = 3.87, Scheffe's $F = 28.032$, 84.426, and 168.353 for h 1-3, respectively: VTA: Fisher's PLSD = 6.478, Scheffe's $F = 18.521$, 82.842, and 96.972 for h 1-3, respectively). Synaptic concentrations of DA were significantly affected in the VTA vis-à-vis the NAcc in the third hour [ANOVA, $F(1, 5) = 21.587, p < 0.0056, N = 5$]. DA in the NAcc and VTA was significantly and positively correlated [Pearson product: $r_{(a)} = 0.927, z = 1.6366, p < 0.01$].

Figure 2(c) shows the temporal effects of SC cocaine at a dose of 40 mg/kg. Cocaine significantly decreased the electrochemical signal for DA in the NAcc and VTA [NAcc: ANOVA, $F(3, 15) = 67.727, p < 0.0001, N = 7$; VTA: ANOVA, $F(3, 15) = 94.316, p < 0.0001, N = 4$]. However, synaptic concentrations of DA in the NAcc and VTA did not continue to decrease further than was seen at the 20-mg/kg dose. Indeed, DA synaptic concentrations, although still significantly decreased from control values, increased above those of the 20-mg/kg dose and showed a trend toward baseline values at the end of the study. Posthoc analysis revealed that DA was significantly decreased in each of the 3 h tested both in the NAcc and VTA (NAcc: Fisher's PLSD = 2.645, Scheffe's $F = 27.080$, 51.384, and 49.708 for h 1, 2, and 3, respectively; VTA: Fisher's PLSD = 4.919, Scheffe's $F = 46.05$, 78.229, and 57.912 for h 1,2, and 3, respectively). The DAergic response to SC cocaine was significantly greater in VTA somatodendrites than in NAcc terminals [ANOVA, $F(1, 5) = 149.906, p < 0.0001, N = 4$-7; $F(1, 5) = 232.192, p < 0.0001, N = 4$-7; $F(1, 5) = 235.343, p < 0.0001, N = 4$-7, h 1-3, respectively]. DA in the NAcc and VTA was significantly and positively correlated [Pearson product: $r_{(a)} = 0.95, z = 1.8318, p < 0.01$]. Cocaine dose dependent effects on DA were also positively and significantly correlated ($r > 0.839, z > 1.2212, p < 0.01$).

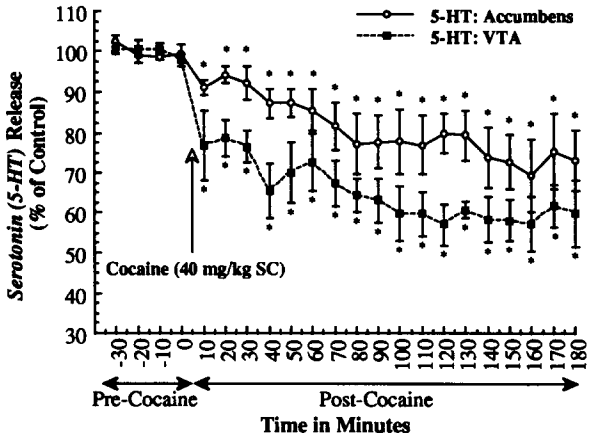
Figures 3a-c show the temporal effects of SC cocaine on synaptic concentrations of 5-HT in the NAcc and VTA in the chloral hydrate-anesthetized paradigm. Figure 3(a) shows that SC cocaine (10 mg/kg) significantly decreased synaptic concentrations of 5-HT in the NAcc and VTA [NAcc: ANOVA, $F(3, 15) = 42.236, p < 0.0001, N = 4$; VTA: ANOVA, $F(3, 15) = 47.866, p < 0.0001, N = 5$]. Posthoc analysis further showed that the significant decreases that occurred in synaptic concentrations of 5-HT took place in every hour tested in both the NAcc and VTA (NAcc: Fisher's PLSD = 4.248, Scheffe's $F = 17.884$, 27.966, and 34.465 for h 1-3, respectively; VTA: Fisher's PLSD = 5.092, Scheffe's $F = 14.095$, 44.425, and 24.351 for h 1-3, respectively). Serotonergic responses to SC cocaine in the VTA were more significantly affected than those in the NAcc. However, statistical significance was reached only in the second hour [ANOVA, $F(1, 5) = 57.71, p < 0.0006, N = 4,5$]. 5-HT in the NAcc and VTA was significantly and positively correlated [Pearson product: $r_{(a)} = 0.821, z = 1.1566, p < 0.01$].



(a)



(b)



(c)

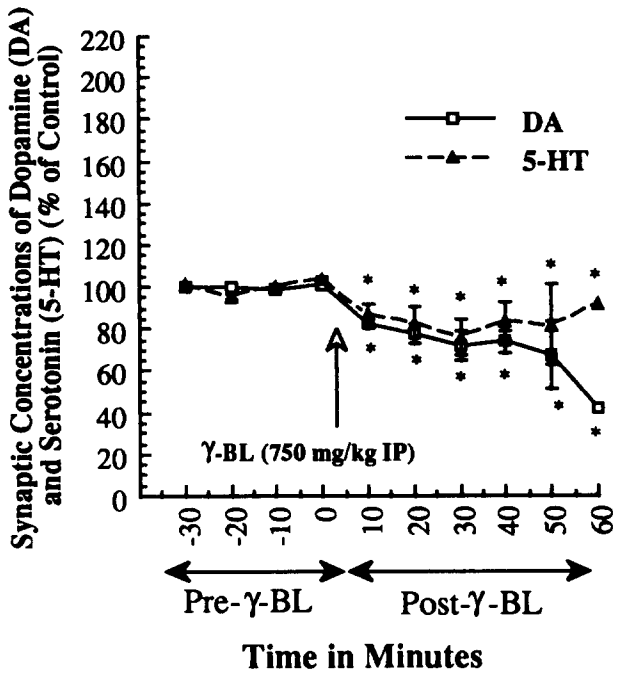
FIG. 3. Effect of SC cocaine on synaptic concentrations of 5-HT in the NAcc and VTA in the chloral hydrate-anesthetized paradigm. (a) 10 mg/kg, $N = 4$-5. (b) 20 mg/kg, $N = 5$. (c) 40 mg/kg, $N = 4$-7. *$p < 0.05$, 95% CL (cf. text for ANOVA statistics).

Figure 3(b) shows that SC cocaine (20 mg/kg) significantly decreased synaptic concentrations of 5-HT in the NAcc and VTA [NAcc: ANOVA, $F(3, 15) = 166.313$, $p < 0.0001$, $N = 5$; VTA: ANOVA, $F(3, 15) = 83.248$, $p < 0.0001$, $N = 5$]. Statistical significance was found in each of the 3 h tested (NAcc: Fisher's PLSD = 3.088, Scheffe's $F = 26.733$, 113.895, and 125.221 for h 1-3, respectively; VTA: Fisher's PLSD = 8.018, Scheffe's $F = 22.252$, 60.286, and 63.89, for h 1-3, respectively). The effects of SC cocaine on synaptic concentrations of 5-HT were significantly greater in VTA vis-à-vis the NAcc in each of the 3 h tested [ANOVA, $F(1, 5) = 62.028$, $p < 0.0005$, $N = 5$; $F(1, 5) = 137.848$, $p < 0.0001$, $N = 5$; $F(1, 5) = 24.45$, $p < 0.0043$, $N = 5$, h 1, 2, and 3, respectively]. 5-HT in the NAcc and VTA was significantly and positively correlated [Pearson product: $r_{(a)} = 0.917$, $z = 1.5698$, $p < 0.01$].

Figure 3(c) shows that SC cocaine (40 mg/kg) decreased 5-HT synaptic concentrations significantly in both the NAcc and VTA [NAcc: ANOVA, $F(3, 15) = 159.088$, $p < 0.0001$, $N = 7$; VTA: ANOVA, $F(3, 15) = 290.17$, $p < 0.0001$, $N = 4$]. Statistical significance was found in each of the 3 h tested (NAcc: Fisher's PLSD = 2.801, Scheffe's $F = 20.741$, 89.351, and 131.915 for h 1-3, respectively; VTA: Fisher's PLSD = 3.29; Scheffe's $F = 99.537$, 202.702, and 231.537 for h 1-3, respectively). The VTA also seems the more vulnerable neuroanatomic substrate vis-à-vis the NAcc insofar as 5-HT is concerned [ANOVA, $F(1, 5) = 174.714$, $p < 0.0001$, $N = 7,4$; $F(1, 5) = 127.817$, $p < 0.0001$, $N = 7,4$; $F(1, 5) = 236.608$, $p < 0.0001$, $N = 7,4$, h 1-3, respectively]. 5-HT in the NAcc and VTA was significantly and positively correlated [Pearson product: $r_{(a)} = 0.936$, $z = 1.7047$, $p < 0.01$]. Cocaine dose dependent effects on 5-HT were also positively and significantly correlated ($r > 0.843$, $z > 1.2212$, $p < 0.01$). Like DA, synaptic concentrations of 5-HT in NAcc and VTA did not decrease further than did the 20 mg/kg dose effect.

Figures 4(a) and 4(b) describe the response of DA and 5-HT neurons in the NAcc to γ-BL alone and in combination with SC cocaine (20 mg/kg). In Figure 4(a), γ-BL significantly decreased the electroactive species for DA [ANOVA, $F(1, 5) = 26.209$, $p < 0.0037$, $N = 7$] for the hour tested. The general anesthetics, γ-BL plus chloral hydrate, were toxic in most animals within approximately 40–50 min after γ-BL injection. One can reasonably assume that the general anesthetic effect of γ-BL (50) and that of chloral hydrate proved a respiratory challenge that could not be overcome in an approximate two thirds of the subpopulation. On the other hand, γ-BL produced a transient but significant decrease in 5-HT [ANOVA, $F(1, 5) = 14.095$, $p < 0.0132$, $N = 7$] that lasted for 30 min and then significantly increased for 30 min [ANOVA, $F(1, 5) = 13.186$, $p < 0.015$, $N = 2$-7].

Figure 4(b) shows that SC cocaine (20 mg/kg) significantly decreased the electroactive species for DA in the presence of a significant (33⅓%, $p < 0.05$, 95% CL) neuronal impulse flow block by γ-BL [ANOVA, $F(1, 5) = 21.675$, $p < 0.0056$, $N = 4$]. However, SC cocaine did not continue to significantly decrease synaptic concentrations of DA further over the significantly decreased DA due to γ-BL alone last ½ h [ANOVA, $F(1, 5) = 0.624$, $p < 0.4654$, $N = 4$-7]. It is important to note, though, that the DA decrease after SC cocaine, which was seen after γ-BL impulse blockade, is a composite of a continuing blockade of DA impulse flow. γ-BL has a long-lasting effect on DA neurons (unpublished data from this laboratory). Too, in this group, data points are diminished toward the 60-min mark because the combination



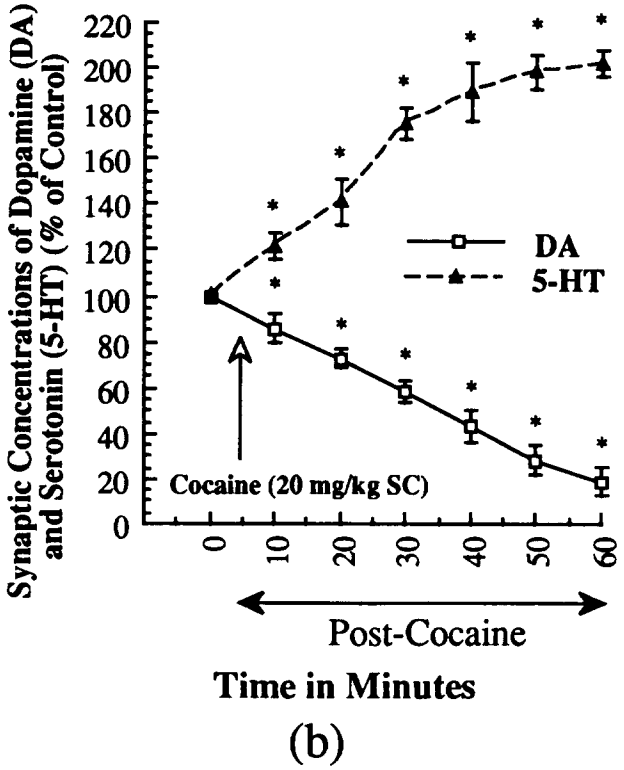


FIG. 4. (a) Effect of γ-BL (750 mg/kg, IP) on synaptic concentrations of DA and 5-HT in the NAcc in the chloral hydrate-anesthetized paradigm ($N = 7$). *$p < 0.05$, 95% CL. (b) (■), 33⅓% inhibition of neuronal impulse flow by γ-BL. Effect of SC cocaine (20 mg/kg) on synaptic concentrations of DA and 5-HT in the NAcc in the chloral hydrate-anesthetized paradigm in a separate group of animals in the presence of a 33⅓% DA impulse flow block. *$p < 0.05$, 95% CL ($N = 4$) (cf. text for ANOVA statistics).

of the general anesthetics, γ-BL and chloral hydrate, was toxic. Furthermore, synaptic concentrations for 5-HT in NAcc were significantly increased [ANOVA, $F(1, 5) = 28.162, p < 0.0032, N = 4–7$] in the presence of a significant 33⅓% neuronal impulse flow inhibition by γ-BL. 5-HT was further and significantly increased after SC cocaine administration over the 5-HT increases due to the presence of γ-BL alone [$F(1, 5) = 14.749, p < 0.0121, N = 4–7$].

DISCUSSION

The present findings show that IV cocaine exerts enhancing effects on DA release in NAcc in the presence of chloral hydrate anesthesia. Taken together with other studies showing comparable IV cocaine–DAergic effects in the NAcc in the freely moving animal paradigm (11,24,40), the data show that the general anesthetic effects of chloral hydrate do not influence the response of DA in the NAcc to IV cocaine. Previous findings have also posited a lack of local anesthetic involvement in the A_{10} response to IV cocaine (16). Too, the present results are in agreement with previous in situ studies, using the anesthetized paradigm, that show that IV cocaine increased extracellular DA concentrations in the NAcc (35) and increased extracellular DA concentrations in both the NAcc and VTA (3). Consistent with the latter study, baseline DA concentrations in the VTA were generally less than those in the NAcc. However, it is noteworthy that the comparison between the two studies is relative, that is, whereas each dialysate sampling (3) of DA is integrated over 20 min, each in vivo electrochemical sampling of DA occurs within 10–15 s. Moreover, the present data are consistent with the literature with respect to the time course effects of IV cocaine (3,35). Indeed, the significantly increased DA release, which remains visible 60 min after IV cocaine administration (1 mg/kg) despite decreased levels of cocaine in the NAcc (37), may help explain the addictive potency of cocaine. Notably, new findings show that 5-HT release is significantly increased in the NAcc after IV cocaine in the chloral hydrate-anesthetized preparation. The 5-HT effect is concurrent and correlated with the DAergic response; this is particularly interesting because somatodendritic cell firing rates in the VTA and DR after cocaine also parallel each other, at least acutely (13,16). The results are in clear agreement with current thinking that cocaine-induced terminal reuptake inhibition (22,45), which would also elevate synaptic DA and 5-HT, can produce a silencing of VTA and DR cell firing.

Nonetheless, in contrast to the suppression of DA cell firing in the VTA and also 5-HT cell firing in the DR, both of which are seen after IV administration of cocaine, the direct microiontophoretic application of cocaine only weakly blocks VTA and DR cell firing (13,16). This discrepancy may indicate further the involvement of a long loop negative feedback interplay. Importantly, though, it may have additional implications, that is, other actions, are involved in the systemic effects of cocaine. Correspondingly, cocaine has long-lived and powerful peripheral vasoconstrictor effects. In fact, cocaine is the only local anesthetic that is a vasoconstrictor (41). Cocaine has been shown to increase the vasoactive sensitivity and/or maximum response of the coronary and femoral artery to other vasoactive substances, NE, 5-HT, and the thromboxane A_2 analog, U-46619 (26). Furthermore, the pharmacokinetic effects of cocaine are highly dependent upon route of administration. Nayak et al. (36) reported that the $t_{1/2}$ of acute IV cocaine is 0.3 and 0.4 h in blood and brain, respectively, whereas the $t_{1/2}$ of acute SC cocaine is 0.8 and 1.0 h in brain and blood, respectively; cocaine is seen in brain 4 h after SC administration (36). Thus, cocaine via the SC route provides longer-lasting effects than the IV route, in part due to local vasoconstriction and in part due to absorptive processes, which are inherently eliminated with IV injection.

Given these dramatic dispositional differences, then, new findings are presented showing that SC cocaine administration caused a dose-dependent decrease in DA and 5-HT release concurrently, in the NAcc and in VTA. The results repeat previous studies showing that the DAergic response to SC cocaine (20 mg/kg) was decreased in the NAcc in the chloral hydrate-anesthetized rat (10). A colocalization of DA and 5-HT was apparent and the decrease in biogenic amine release, coinduced by cocaine, was highly correlative. The neuroanatomic substrate VTA appears more vulnerable than does the NAcc to the biogenic amine effects of SC cocaine, although the NAcc was significantly affected. However, perhaps most contributory from the present findings is the demonstration that "route of administration" of cocaine significantly affects the neurochemical DA and 5-HT response to cocaine. The data lend an explanatory note to previous data showing that the route of administration of cocaine, even on an acute basis, influences behavior differently (31) and that even the pattern of cocaine administration within any one route may evoke differential responses (29). Importantly, if one can assume identical cell firing effects with both SC and IV cocaine in the VTA and DR, the present data reveal that increased synaptic concentrations of DA and 5-HT at the neuronal terminal may not be necessary for, or are uncoupled with, cocaine's action on somatodendrites.

Importantly, these findings show that the SC route of administration significantly decreased the cocaine-induced response to DA release in the NAcc and VTA. Taken together with other data (7,9), which show that SC cocaine increased DA release in the NAcc and VTA, the present data indicate that cocaine-induced effects on mesoaccumbens DA release are subject to the general anesthetic effects previously described (18,28). The data are supported clinically; surgery is complicative in the cocaine-addicted patient (17). Moreover, the general anesthetic effects that influence the DAergic reaction to SC cocaine in the anesthetized paradigm do not cross over to the 5-HTergic response in DA pathways in the conscious paradigm (7).

Provocatively, the present results may be explained as the biogenic aminergic response to the formation of a cocaine metabolite and not to cocaine. Because the disposition of SC cocaine is long lived, further opportunity is provided for metabolite derivation. One cocaine metabolite, benzoylecgonine, itself is a potent vasoconstrictor with vasoactive properties greater than NE and cocaine (33). Interestingly, benzoylecgonine does not increase reinforcement behavior and also does not displace [^{3}H]cocaine binding (46). Thus, it is possible that the action of SC cocaine in the chloral hydrate-anesthetized rat paradigm is due to the formation of benzoylecgonine.

Finally, presynaptic DA and 5-HT release mechanisms for cocaine are evidenced here because release mechanisms are dependent upon impulse flow and cocaine's actions on synaptic concentrations of DA and 5-HT in the NAcc were inhibited when impulse flow was blocked with γ-BL. SC cocaine did not further significantly alter synaptic concentrations of DA in the NAcc in the presence of neuronal impulse flow inhibition with γ-BL. Therefore, other data that show that available synaptic DA is necessary for the action of cocaine (8,12) are supported. The data agree with another report showing that cocaine does not reverse γ-BL-altered DAergic function (19).

Also, the evidence presented for a presynaptic 5-HTergic releasing mechanism is in agreement with a previous report (8). The evidence is more conclusive here for 5-HT than for DA because the electroactive species for 5-HT is exactly subtended when neuronal impulse flow is blocked. Alternatively, the explanation for the 5-HTergic effects of cocaine after γ-BL, may be simply that the cocaine-induced 5-HT reuptake inhibitory effects are seen after release mechanisms are blocked.

In summary, the data show a) that IV cocaine effects on DA and 5-HT release are not influenced by anesthesia, b) that IV and SC cocaine differ in their resultant neurochemical effects on DA and 5-HT release, and c) that the effect of SC cocaine on DA release is influenced by chloral hydrate anesthesia. Interestingly, acute SC cocaine administration may provide a unique acute animal model for the study of DA depletion by chronic cocaine and/or its metabolites.

ACKNOWLEDGEMENTS

This research was supported by National Institute on Drug Abuse Award R01-04755-01 and Professional Staff Congress/CUNY Awards RF 661188, 669201, and DHHS PHS Grant 2-S07-RR07132 to P.A.B. The author thanks Frankie Eng, Frank T. Phelan, and Robert T. Wechsler for excellent technical assistance.

REFERENCES

1. Azmitia, E. C.; Segal, M. An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. J. Comp. Neurol. 179:641–667; 1978.
2. Blaha, C. D.; Jung, M. E. Electrochemical evaluation of stearate-modified graphite paste electrodes: Selective detection of dopamine is maintained after exposure to brain tissue. J. Electroanal. Chem. 310:317–334; 1991.
3. Bradberry, C. W.; Roth, R. H. Cocaine increases extracellular dopamine in rat nucleus accumbens and ventral tegmental area as shown by in vivo microdialysis. Neurosci. Lett. 103:97–102; 1989.
4. Broderick, P. A. Striatal neurochemistry of dynorphin-(1-13): In vivo electrochemical semidifferential analyses. Neuropeptides 10: 369–386; 1987.
5. Broderick, P. A. Characterizing stearate probes in vitro for the electrochemical detection of dopamine and serotonin. Brain Res. 495:115–121; 1989.
6. Broderick, P. A. State-of-the-art microelectrodes for in vivo voltammetry. Electroanalysis 2(3):241–251; 1990.
7. Broderick, P. A. Cocaine-on-line analysis of an accumbens amine neuronal basis for psychomotor behavior. Pharmacol. Biochem. Behav. 40:959–968; 1991.
8. Broderick, P. A. In vivo voltammetric studies on release mechanisms for cocaine with γ-butyrolactone. Pharmacol. Biochem. Behav. 40:969–975; 1991.
9. Broderick, P. A. Cocaine's colocalized effects on synaptic serotonin and dopamine in ventral tegmentum in a reinforcement paradigm. Pharmacol. Biochem. Behav. 42:889–898; 1992.
10. Broderick, P. A.; Phelan, F. T. An in vivo voltammetric analysis of dopamine release in nucleus accumbens: I. Morphine effects. II. Cocaine effects. Adv. Biosci. 75:209–212; 1989.
11. Brown, E. E.; Finlay, J. M.; Wong, J. T. F.; Damsma, G.; Fibiger, H. C. Behavioral and neurochemical interactions between cocaine and buprenorphine: Implications for the pharmacotherapy of cocaine abuse. J. Pharmacol Exp. Ther. 256:119–126; 1991.
12. Carboni, E.; Imperato, A.; Perezzani, L.; DiChiara, G. Amphetamine, cocaine, phencyclidine and nomifensine increase extracellular dopamine concentrations preferentially in the nucleus accumbens of freely moving rats. Neuroscience 28:653–661; 1989.
13. Cunningham, K. A.; Lakoski, J. M. Electrophysiological effects of cocaine and procaine on dorsal raphe serotonin neurons. Eur. J. Pharmacol. 148:457–462; 1988.
14. Dahlstrom, A.; Fuxe, K. Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. Acta Physiol. Scand. 62(suppl. 232):1–55; 1964.
15. deWit, H.; Wise, R. A. Blockade of cocaine reinforcement in rats with the dopamine receptor blocker pimozide but not with the noradrenergic blockers phentolamine or phenoxybenzamine. Can. J. Psychol. 31:195–203; 1977.
16. Einhorn, L. C.; Johansen, P. A.; White, F. J. Electrophysiological effects of cocaine in the mesoaccumbens dopamine system: Studies in the ventral tegmental area. J. Neurosci. 8:100–112; 1988.
17. Elliott, H. W. Effects of street drugs on anesthesia. Int. J. Clin. Pharmacol. 12:134–140; 1975.
18. Freeman, A. S.; Bunney, B. S. Activity of A_9 and A_{10} dopaminergic neurons in unrestrained rats: Further characterization and effects of apomorphine and cholecystokinin. Brain Res. 405:46–55; 1987.
19. Galloway, M. P. Regulation of dopamine and serotonin synthesis by acute administration of cocaine. Synapse 6:63–72; 1990.
20. Gawin, F. H.; Allen, D.; Humblestone, B. Outpatient treatment of "crack" cocaine smoking with flupenthixol decanoate. A preliminary report. Arch. Gen. Psychiatry 46:322–325; 1989.
21. Gelbert, M. B.; Curran, D. J. Alternating current voltammetry of dopamine and ascorbic acid at carbon paste and stearic acid modified carbon paste electrodes. Anal. Chem. 58:1028–1032; 1986.
22. Heikkila, R. E.; Orlansky, H.; Cohen, G. Studies on the distinction between uptake inhibition and release of [^{3}H]dopamine in rat brain tissue slices. Biochem. Pharmacol. 24:847–852; 1975.
23. Herve, D.; Pickel, V. M.; Joh, T. H.; Beaudet, A. Serotonin axon terminals in the ventral tegmental area of the rat: Fine structure and synaptic input to dopaminergic neurons. Brain Res. 435: 71–83; 1987.
24. Hurd, Y. L.; Kehr, J.; Ungerstedt, U. In vivo microdialysis as a technique to monitor drug transport; correlation of extracellular cocaine levels and dopamine overflow in the rat brain. J. Neurochem. 51:1314–1316; 1988.
25. Johanson, C. E.; Fischman, M. W. The pharmacology of cocaine related to its abuse. Pharmacol. Rev. 41:3–52; 1989.
26. Jones, L. F.; Tackett, R. L. Chronic cocaine treatment enhances the responsiveness of the left anterior descending coronary artery and the femoral artery to vasoactive substances. J. Pharmacol. Exp. Ther. 255:1366–1370; 1990.
27. Kalivas, P. W.; Duffy, P. Effect of acute and daily cocaine treatment on extracellular dopamine in the nucleus accumbens. Synapse 5:48–58; 1990.
28. Kelland, M. D.; Freeman, A. S.; Chiodo, L. A. Chloral hydrate anesthesia alters the responsiveness of identified midbrain dopamine neurons to dopamine agonist administration. Synapse 3:30–37; 1989.
29. King, C. E.; Joyner, C.; Lee, T.; Kuhn, C.; Ellinwood, E. H., Jr. Intermittent and continuous cocaine administration: Residual behavioral states during withdrawal. Pharmacol. Biochem. Behav. 43:243–248; 1992.
30. Kosten, T. R. Pharmacotherapeutic interventions for cocaine abuse. Matching patients to treatments. J. Nerv. Ment. Dis. 177: 379–389; 1989.
31. Lau, C. E.; Imam, A.; Ma, F.; Falk, J. L. Acute effects of cocaine on spontaneous and discriminative motor functions: Relation to route of administration and pharmacokinetics. J. Pharmacol. Exp. Ther. 257:444–456; 1991.
32. Loh, E. A.; Roberts, D. C. Break-points on a progressive ratio schedule reinforced by intravenous cocaine increase following depletion of forebrain serotonin. Psychopharmacology (Berl.) 101:262–266; 1990.

33. Madden, J. A.; Powers, R. H. Effect of cocaine and cocaine metabolites on cerebral arteries in vitro. Life Sci. 47:1109–1114; 1990.
34. May, L. J.; Wightman, R. M. Effects of D_2 antagonists on frequency-dependent stimulated dopamine overflow in nucleus accumbens and caudate-putamen. J. Neurochem. 53:898–906; 1989.
35. Moghaddam, B.; Bunney, B. S. Differential effect of cocaine on extracellular dopamine levels in rat medial prefrontal cortex and nucleus accumbens: Comparison to amphetamine. Synapse 4: 156–161; 1989.
36. Nayak, P. K.; Misra, A. L.; Mule, S. J. Physiological disposition and biotransformation of [^{3}H]cocaine in acutely and chronically treated rats. J. Pharmacol. Exp. Ther. 196:556–569; 1976.
37. Pan, H. T.; Menacherry, S.; Justice, J. B., Jr. Differences in the pharmacokinetics of cocaine in the naive and cocaine experienced rat. J. Neurochem. 56:1299–1306; 1991.
38. Parent, A.; Descarries, L.; Beaudet, A. Organization of ascending serotonin systems in the adult rat brain. A radioautographic study after intraventricular administration of [^{3}H]5-hydroxytryptamine. Neuroscience 6:115–138; 1981.
39. Pelligrino, L. J.; Cushman, A. J. A stereotaxic atlas of the rat brain. New York: Appleton-Century-Crofts; 1967.
40. Pettit, H. O.; Justice, J. B., Jr. Dopamine in the nucleus accumbens during cocaine self-administration as studied by in vivo microdialysis. Pharmacol. Biochem. Behav. 34:899–904; 1989.
41. Ritchie, J. M.; Greene, N. M. Local anesthetics. In: Gilman, A. A.; Rall, T. W.; Nies, A. S.; Taylor, P., eds. The pharmacological basis of therapeutics. 8th ed. Elmsford, NY: Pergamon Press; 1990:311–331.
42. Ritz, M. C.; Lamb, R. J.; Goldberg, S. R.; Kuhar, M. J. Cocaine receptors on dopamine transporters are related to self-administration of cocaine. Science 237:1219–1223; 1987.
43. Roberts, D. C. S.; Koob, G. F. Disruption of cocaine self-administration following 6-hydroxydopamine lesions of the ventral tegmental area in rats. Pharmacol. Biochem. Behav. 17:901–904; 1982.
44. Roberts, D. C. S.; Koob, G. F.; Klonoff, P.; Fibiger, H. C. Extinction and recovery of cocaine self-administration following 6-hydroxydopamine lesions of the nucleus accumbens. Pharmacol. Biochem. Behav. 12:781–787; 1980.
45. Ross, S. B.; Renyi, A. L. Inhibition of the uptake of tritiated 5-hydroxytryptamine in brain tissue. Eur. J. Pharmacol. 7:270–277; 1969.
46. Spealman, R. D.; Madras, B. K.; Bergman, J. Effects of cocaine and related drugs in nonhuman primates. II. Stimulant effects on schedule-controlled behavior. J. Pharmacol. Exp. Ther. 251:142–149; 1989.
47. Steinbusch, H. W. M. Distribution of serotonin-immunoreactivity in the central nervous system of the rat-cell bodies and terminals. Neuroscience 6:557–618; 1981.
48. Taber Pierce, E.; Foote, W. E.; Hobson, J. A. The efferent connection of the nucleus raphe dorsalis. Brain Res. 107:137–144; 1976.
49. Walters, J. R.; Roth, R. H. Dopaminergic neurons: Drug-induced antagonism of the increase in tyrosine hydroxylase activity produced by cessation of impulse flow. J. Pharmacol. Exp. Ther. 191:82–91; 1974.
50. Winters, W. D.; Spooner, C. E. Various seizure activities following gamma-hydroxybutyrate. Int. J. Neuropharmacol. 4:197–200; 1965.