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Effects of Cocaine on Release and Uptake of Dopamine In Vivo: Differentiation by Mathematical Modeling

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NICOLAYSEN, L. C. AND J. B. JUSTICE, JR. *Effects of cocaine on release and uptake of dopamine in vivo: Differentiation by mathematical modeling.* PHARMACOL BIOCHEM BEHAV 31(2) 327–335, 1988.—Although considerable effort has been invested trying to distinguish between the effects of cocaine on dopamine (DA) uptake and release in both in vitro and in vivo experiments, disagreement over the specific actions of cocaine remains. The results obtained by combining experimental extracellular DA data with a mathematical model of the dopaminergic neuron allow examination of the cocaine uptake inhibition/release question. The extracellular DA concentration profile observed following a 30 mg/kg IP cocaine injection can be modeled if both pre- and postsynaptic uptake are competitively inhibited by cocaine with or without an enhanced DA release effect. However, if cocaine elicits enhanced DA release, modeling predicts a 40% increase over basal levels of 3,4-dihydroxyphenylacetic acid (DOPAC) and a 30% increase in homovanillic acid (HVA) at 60 minutes following a 30 mg/kg IP cocaine injection. Reported DOPAC and HVA data for similar cocaine doses indicate little change in either DOPAC or HVA. These data agree best with modeled metabolite predictions for little or no cocaine-enhanced DA release.

Computer simulation Rat Striatum Pharmacokinetics Metabolism DOPAC HVA

COCAINE has been shown to increase extracellular dopamine in vivo in the rat striatum (7, 33, 39). Many of cocaine's effects on the central nervous system have been attributed to its ability to inhibit the uptake of dopamine (12, 17, 20, 22, 23, 45), its ability to enhance dopamine release (3, 4, 6, 10, 11, 18, 25), or a combination of both (1, 2, 37, 46). The difficulty associated with unequivocally distinguishing between uptake and release in both in vitro and in vivo experiments has caused considerable disagreement over the specific neurochemical actions of cocaine.

Cocaine has also been shown to inhibit the uptake of other monoamines including serotonin (15) and norepinephrine (24). However, the self-administration reward aspects of cocaine are thought to be primarily associated with the dopaminergic system (8, 16, 42, 43, 55, 57). The cocaine receptor associated with substance abuse has been proposed to be the one related to dopamine uptake inhibition (41). Thus, the responses of the dopaminergic system to cocaine are of particular interest.

Approaches taken to address the uptake inhibition/release issue usually involve monitoring the accumulation and/or release of radiolabeled dopamine in vitro by synaptosomes or tissue slices. Experiments involving synaptosomes only give information concerning the presynaptic nerve terminal. Two dopamine uptake pathways have been reported, one of which is presynaptic (23,28). It has been suggested that more than just a presynaptic uptake inhibition effect of cocaine should be addressed (38).

The time course of both extracellular dopamine (7) and extracellular cocaine (32) in the striatum following a single 30 mg/kg IP injection of cocaine have been measured by in vivo microdialysis techniques. Extracellular cocaine and dopamine have been found to be linearly related in the striatum (32). The combination of this data with a mathematical model of the dopaminergic nerve terminal (21) is suggested as a new approach to addressing the cocaine uptake inhibition/release question. The modeling concepts presented previously are used in the present work to make predictions regarding experimentally verifiable consequences of dopamine uptake inhibition and enhancement of release. These predictions are compared with data from the literature.

METHOD

Computer simulation and simplex optimization are used to develop several models for cocaine's neurochemical actions on the dopaminergic nerve terminal in the rat striatum (21). The modeling methodology employed here allows one

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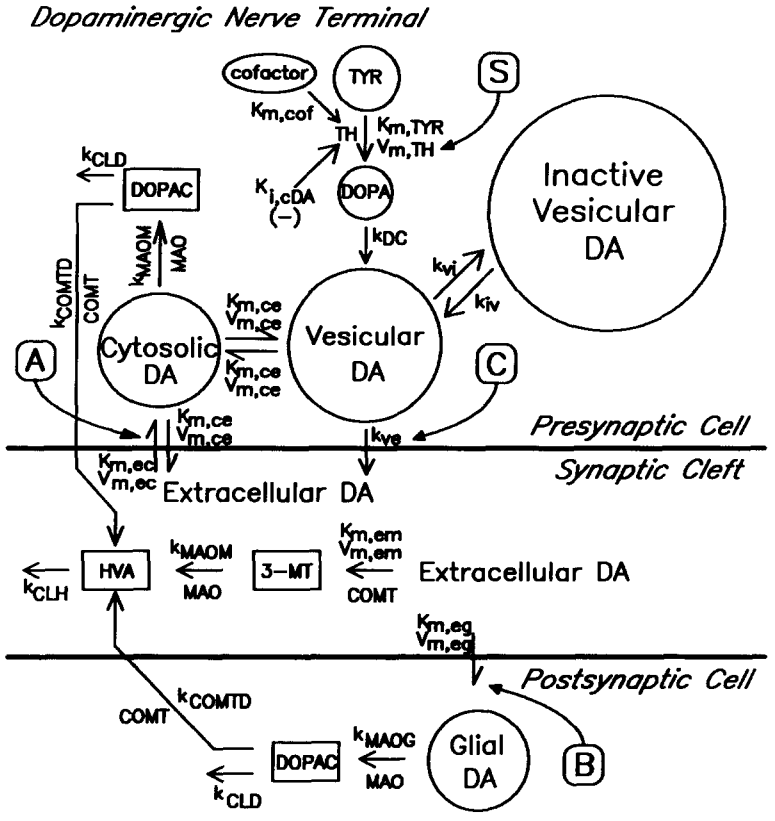

FIG. 1. Diagram of the dopaminergic nerve terminal used for modeling. Table 1 contains the descriptions, values, and origin of the constants and parameters for the model shown here. The capital letters enclosed in rounded squares point to the processes postulated to be affected by cocaine. A refers to the inhibition of presynaptic uptake of DA, B to inhibition of postsynaptic uptake of DA, C to cocaine enhanced vesicular DA release, and S to cocaine effects on DA synthesis.

to develop and test hypothesized representations and manipulations of the dopaminergic nerve terminal in a rational, systematic, and quantitative manner.

Model of the Dopaminergic Nerve Terminal

The model was developed by integrating data on dopamine (DA) synthesis, compartmentation, release, uptake, and metabolism from the literature on DA, and where possible, DA in the rat striatum, as previously reported (21). The structure of the model considered here is diagramed in Fig. 1.

The parameters of the model are listed in Table 1. Values are either taken directly or derived from the literature or have been determined by the simplex optimization of the model to agree with data using modeling methodology developed in our laboratory (21). The data used for optimization of the values of unknown parameters of the DA nerve terminal model included steady state passage of radioactivity, total DA at steady state, and change in extracellular DA concentration during electrical stimulation. These data represent a very wide dynamic range of responses. The optimized model is robust in that it reproduces the data satisfactorily for the steady state, under slowly changing conditions, and under conditions of very rapid change. Optimization by the sequential simplex method used here involves minimization of the error, the squared difference between the model and data, by manipulating a set of parameters to obtain the best agreement between the model and that data. Values of parameters are determined by the optimization process in the development of the model. A second optimization is then performed to determine the value of the parameters used to model the postulated effects of cocaine.

Two DA uptake mechanisms are reported to exist in vivo (23,28). Sodium-dependent cocaine binding sites associated with high affinity DA uptake are located presynaptically (23,28). Low affinity DA uptake is not presynaptic, located either elsewhere on the nerve terminal or extraneuronally (i.e., glial cells) (28). Cultured glial cells exhibit low affinity high capacity DA uptake (36,56). In the model, a high affinity, low capacity uptake process maintains extracellular DA at a low concentration (47). A postsynaptic low affinity, high capacity uptake process also clears DA from the extracellular fluid.

Development of the model has deliberately excluded receptor mediated regulation of synthesis. End product synthesis inhibition by cytosolic DA is included in the term for tyrosine hydroxylase activity, but receptor mediated regulation is omitted. This does not imply that receptor mediated regulation of synthesis is unimportant. The effect of cocaine on the DA synthesis rate has been studied (34) and is included directly to account for the synthesis rate attenuation effects of cocaine.

A maximum total DA uptake rate of 7.1 μM/sec has been

TABLE 1

CONSTANTS AND PARAMETERS OF MODEL OF THE DOPAMINERGIC NEURON

	Description	Value	Unit	Reference
Constant				
Cofactor	Tetrahydrobiopterin, TH Cofactor	3.0	nmol/g	(25)
TYR	Tyrosine, Precursor of DOPA	150.0	nmol/g	(13)
Parameter				
$V_{m,TH}$	V_{max} for TH	50.0	nmol/g/min	53*
$K_{m,TYR}$	K_m for TYR	55.3	nmol/g	30
$K_{i,cDA}$	K_i for free cytosolic DA (cDA)	110.0	nmol/g	30
$K_{m,cof}$	K_m for TH cofactor	910.0	nmol/g	30
k_{DC}	k for DOPA decarboxylase (DC)	1.38	min^{-1}	52
$K_{m,cv}$	K_m cDA to vesicular DA (vDA)	0.298	nmol/g	47†
$V_{m,cv}$	V_{max} cDA to vDA	0.852	nmol/g/min	47†
$K_{m,vc}$	K_m vDA to cDA	1.03	nmol/g	47†
$V_{m,vc}$	V_{max} vDA to cDA	1.27	nmol/g/min	47†
$K_{m,ce}$	K_m cDA to extracellular DA (eDA)	16.3	nmol/g	47†
$V_{m,ce}$	V_{max} cDA to eDA	15.1	nmol/g/min	47†
$K_{m,ec}$	K_m eDA to cDA	0.159	μM	47‡
$V_{m,ec}$	V_{max} eDA to cDA	8.13	nmol/g/min	47†
k_{iv}	k inactive bound DA (iDA) to vDA	0.568	min^{-1}	Simplex Optimization
k_{vi}	k vDA to iDA	2.34	min^{-1}	Simplex Optimization
k_{ve}	k vDA to eDA, release rate	6.76×10^{-4}	min^{-1}	Simplex Optimization
$K_{m,eg}$	K_m eDA to glial DA (gDA)	15.8	μM	Simplex Optimization
$V_{m,eg}$	V_{max} eDA to gDA	76.5	nmol/g/min	27§
$K_{m,em}$	K_m eDA to 3-MT	0.012	nmol/g	54#
$V_{m,em}$	V_{max} eDA to 3-MT	0.08	nmol/g/min	54#
k_{MAOC}	k cDA to DOPAC	0.0518	min^{-1}	Simplex Optimization
k_{MAOG}	k gDA to DOPAC by MAO	0.0518	min^{-1}	Simplex Optimization
k_{MAOM}	k 3-MT to HVA by MAO	0.302	min^{-1}	54
k_{COMTD}	k DOPAC to HVA by COMT	0.038	min^{-1}	9
k_{CLD}	k clearance of DOPAC	0.017	min^{-1}	9
k_{CLH}	k clearance of HVA	0.075	min^{-1}	9

*V_{max} for TH, $V_{m,TH}$, chosen to give synthesis rate of 20 nmol/g/hr (53).

†Value converted from pmol/mg protein in synaptosomes to nmol/g tissue units using a 0.065 conversion factor (V. J. Nickolson, personal communication, 1987).

‡For use in the model, this value was converted from μM to nmol/g based on 20% extracellular volume (31).

§$V_{m,eg}$ calculated from 7.05 μM/sec total uptake observed following electrical stimulation (27) 84.6 nmol/g/min‡ − 8.1 nmol/g/min for $K_{m,ec}$.

#K_m chosen similar to steady state concentration of extracellular DA and V_{max} chosen to give 3-MT levels similar to those reported (54).

previously determined from uptake following electrical stimulation (27). From this rate, the postsynaptic uptake V_{max} was calculated to be 76.5 nmol/g/min (84.6 nmol/g/min total −8.13 nmol/g/min presynaptic). At steady state in the model, 87.5% extracellular DA is removed by presynaptic uptake, 10.9% by postsynaptic uptake, and the remaining 1.6% by extraneuronal metabolism to 3-MT.

The letters enclosed in rounded rectangles in Fig. 1 indicate the processes postulated to be affected by cocaine. Both presynaptic and postsynaptic uptake processes are illustrated in Fig. 1 and both are examined to determine the extent of their involvement in the effect of cocaine on the dopaminergic system. A refers to the inhibition of presynaptic high affinity uptake of DA, B to the inhibition of postsynaptic low affinity DA uptake. Vesicular DA release has been reported to be increased by cocaine (3). C in Fig. 1 refers to enhanced DA release. The reduction of synthesis is represented by S in Fig. 1.

The DA model presented in Fig. 1 with parameter values summarized in Table 1 was optimized with respect to diverse experimental data as described previously (21). These values remain fixed. The values of the parameters associated with

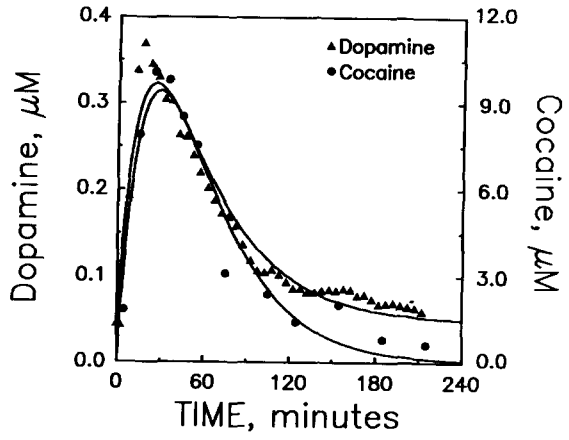

FIG. 2. Data and fitted curves for extracellular dopamine (7) and extracellular cocaine (32) in the rat striatum following a single 30 mg/kg IP injection of cocaine. Cocaine data is shown as circles, and the dashed line is the curve fitted to the data, described by Eqn. 1. The triangles are dopamine data and the solid line is the corresponding fitted curve, described by Eqn. 2.

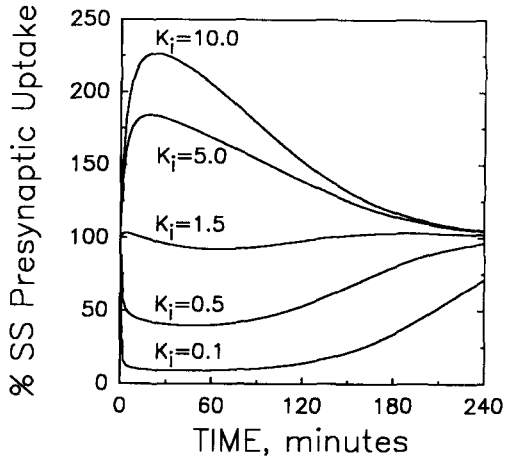

FIG. 3. Percent of steady state presynaptic uptake rate for various values of K_i (μM) for cocaine. Curves were generated using equation for competitive uptake inhibition (Eqn. 3), the concentration of dopamine and cocaine (Eqn. 1 and 2) taken from curves in Fig. 2, and a K_m for presynaptic dopamine uptake of 0.159 μM. Cocaine, which has a K_i of about 1.5 μM, only changes the rate of presynaptic uptake a small amount. Similar curves are obtained for the postsynaptic uptake rate (K_m=15.8 μM).

the effects of cocaine are then optimized (a different optimization) to give agreement between the model and experimental data on extracellular DA response to cocaine for all possible combinations of cocaine effects. Comparison of the different combinations of cocaine effects, and their different neurochemical ramifications, allows one to determine which cocaine effect or combination of effects, are possibly associated with the observed DA response. As described below, the values of the parameters involved in each proposed effect, or combination of effects, are changed until the best possible agreement with the data on extracellular DA is obtained. Following the optimization, these parameter values are used to model the effects of cocaine on the metabolites DOPAC and HVA, and on total DA.

Extracellular Dopamine and Cocaine Data

Data have previously been obtained by in vivo microdialysis for extracellular DA (7) and cocaine (32) in the rat striatum following 30 mg/kg IP dose of cocaine. These data are fitted to simple pharmacokinetic equations to obtain extracellular DA and cocaine concentrations as a function of time for the 30 mg/kg IP dose. The data and resulting fitted curves are shown in Fig. 2. The equations for the curves are as follows:

Eqn. 1 $\text{Cocaine, } \mu\text{M } (t) = 108\ (e^{-0.0304t} - e^{-0.0386t})$

Eqn. 2 $\text{Dopamine, } \mu\text{M } (t) = 0.816\ (e^{-0.0228t} - e^{-0.0593t}) + 0.0465$

where time (t) is in minutes.

Cocaine competitively inhibits DA uptake (12,51). The equation for the presynaptic uptake process, including competitive DA uptake inhibition by cocaine, is as follows:

Eqn. 3 $\text{Uptake Rate} = V_{max,ec}/(1 + (K_{m,ec}/[DA])\ (1 + [\text{cocaine}]/K_{iA}))$

Values for cocaine's inhibition constant K_{iA} have been determined and range from 0.4 to 3.5 μM (5, 12, 17, 19, 22, 29, 40, 41, 45, 48, 50). The mean±standard error of the mean (SEM) of these values is 1.5±0.3 μM (n=11). Figure 3 shows the effect of various values of K_i for cocaine on the rate of presynaptic uptake. This is expressed as a percent of the steady state presynaptic uptake rate for the parameters used. These curves are generated using Eqn. 3 for competitive kinetic inhibition, values for the concentrations of DA and cocaine taken from the fitted curves in Fig. 2 (Eqn. 1 and 2), and a K_m for DA uptake of 0.159 μM (47). This K_m is used in the model for the presynaptic DA uptake process. Values for K_i shown in Fig. 3 are expressed in μM units. Similar results are obtained for postsynaptic uptake inhibition using a K_m of 15.8 μM (data not shown), except larger increases in postsynaptic uptake rate are obtained for the larger K_i values.

Simplex Optimization of Cocaine Effects on Extracellular Dopamine

The values of the parameters used to model cocaine effects are optimized with respect to the extracellular DA data of Fig. 2 for all possible combinations of presynaptic uptake inhibition, postsynaptic uptake inhibition, and enhanced releasing effects of cocaine. The model illustrated in Fig. 1, with the parameters summarized in Table 1, is used to examine specific cocaine effects. The DA data in Fig. 2 was collected at 5 minute intervals with on line in vivo microdialysis (7). The optimization process minimizes the error, taken as the squared difference between the extracellular DA in the model and in vivo data on extracellular DA, corresponding to the points in time for which the data was measured. The midpoint of the 5 minute sampling interval is used to assign points in time for the extracellular DA measurements to be compared to the modeled response. The error is only calculated at times for which there is data, and each data point is assigned equal weight in the total error calculated for a set of parameters. Parameters K_{iA} (presynaptic inhibition), K_{iB} (postsynaptic inhibition), and enhanced release, are deter-

TABLE 2

OPTIMIZATION OF MODEL OF DOPAMINERGIC NEURON FOR THE EFFECTS OF COCAINE ON EXTRACELLULAR DA

Case	K_{iA} μM	K_{iB} μM	Enhanced Release Times [COC]*	Error	Description
S	—	—	—	780.0	synthesis effect only
A	0.013	—	—	432.3	k_i presynaptic only
B	—	0.20	—	322.2	k_i postsynaptic only
C	—	—	2.7	238.7	release only
AB	1.48	1.79	—	28.7	k_is independent
AC	0.011	—	14.0	93.3	k_i presynaptic, release
BC	—	3.44	9.7	64.5	k_i postsynaptic, release
ABC	1.18	4.21	3.0	25.8	k_is independent, release
AA	1.66	1.66	—	28.9	k_is same
AAC	1.82	1.82	0.57	28.1	k_is same, release

Note: CASE S, reduction in synthesis, is included in all cases.
*[COC] is shown in Fig. 2.

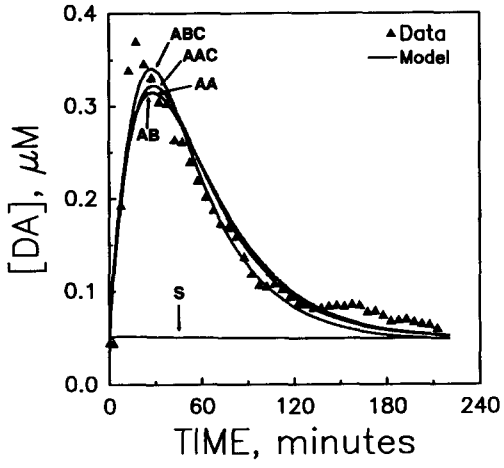

FIG. 4. Results of optimization cases with lowest error for cocaine's effect on extracellular DA for the model of the dopaminergic neuron. The data (triangles) were used to optimize the parameters for each case. Results for the optimized parameters and the error for all cases are summarized in Table 2. S shows the negligible effect of synthesis reduction alone on extracellular DA. To obtain acceptable agreement between the model and the data, enhanced release is not required. Optimizing the two K_is independently does not appreciably improve the agreement between the model and data for cases with or without release.

mined by this optimization process for all combinations of cocaine effects (A, B, and C). The effect of cocaine on DA synthesis is the same for all cases.

The possible combinations of cocaine effects are summarized in Table 2. All cases include the synthesis reduction effect of cocaine. The cocaine effect of presynaptic uptake inhibition (K_{iA}) alone is case A, postsynaptic uptake inhibition (K_{iB}) alone is case B, and enhanced vesicular DA release alone is case C. Case AB is uptake inhibition by cocaine both pre- and postsynaptically where the K_is are allowed to vary independently, and AA where they are forced to be identical. Cases AC and BC are pre- and postsynaptic uptake inhibition, respectively, in conjunction with enhanced DA release. Case ABC is enhanced DA release coupled with uptake inhibition both pre- and postsynaptically where the K_is are obtained independent of each other, and AAC where they are both the same. Obtaining the inhibition constants in this manner allows determination of those values of the constants that result in the best agreement between the model and the data. Published values for K_i could have been chosen, but optimizing the model to obtain them gives further validity to the model if the values obtained are comparable to those reported, as seen later.

An acute dose of 18 mg/kg (IP) cocaine has been shown to reduce tyrosine hydroxylase (TH) activity to about 70% of basal levels at maximum effect (34). The maximum effect is observed 60 minutes postinjection. The model used a synthesis rate attenuated to 50% of steady state levels by 30 mg/kg cocaine. This is an estimate, a larger synthesis reduction was used corresponding to a larger cocaine dose. The time of cocaine's maximum effect on synthesis and the time of maximum cocaine concentration are similar. Cocaine measured in the striatum maximizes between 30 and 40 minutes postinjection (32). Synthesis reduction is therefore assumed to coincide in time with striatal cocaine in the model. In the model, the synthesis effect alone on extracellular DA is minimal, as shown in Fig. 4. However, synthesis reduction does occur and is therefore included in all cases examined.

The fitted cocaine curve from Fig. 2, defined in Eqn. 1, describes the concentration of cocaine used to model competitive inhibition both pre- and postsynaptically. To model enhanced DA release, the vesicular release rate constant is multiplied by the product of the cocaine concentration and the enhanced release parameter. Enhanced release is therefore assumed to be linearly related to extracellular cocaine concentration in the striatum.

RESULTS

The extracellular DA data (7), shown as triangles in Fig. 4, are used to optimize the parameters (K_{iA}, K_{iB}, and enhanced release) for each case as described in the Method section and elsewhere (21). The results of all optimizations of the possible combinations of cocaine effects are summarized in Table 2. The cases with lowest error, cases AA, AB, AAC, and ABC, are illustrated in Fig. 4. These four cases

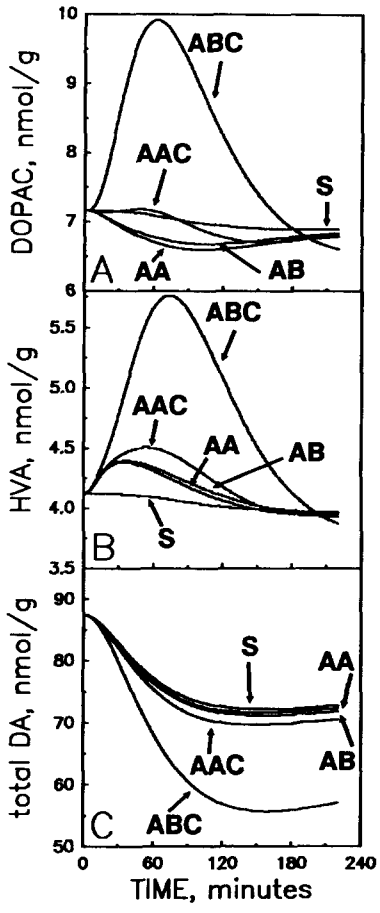

FIG. 5. Modeled responses of DOPAC, HVA, and total DA for the optimized cases shown in Fig. 4. Only case ABC, with significantly enhanced release (30 times the basal release rate at maximum), shows a difference from the other cases for these measurements. The model predicts that a readily detectable change in DOPAC, HVA, and total DA should be seen if cocaine elicits significant enhanced release in the rat striatum.

showed the best agreement (least error) between experimental data and modeled extracellular DA. The reduction of synthesis effect alone, labeled S in Fig. 4, has only a negligible effect on extracellular DA in the model.

Cases with only a single cocaine effect on the DA nerve terminal fail to give agreement with the data (presynaptic uptake inhibition alone, case A; postsynaptic uptake inhibition alone, case B; or enhanced release alone, case C). The error for these cases is quite large when compared to the other possible cases. The validity of cases A and B is also questionable since the values obtained for K_{iA} (0.013 μM) and K_{iB} (0.20 μM) are significantly lower than any reported values for cocaine inhibition of DA uptake.

When release is included with either pre- or postsynaptic uptake inhibition (cases AC and BC) the agreement of the model with the data is substantially better (errors 93.3 and 64.5, respectively). The value of the inhibition constant K_{iA} (0.011 μM) for case AC is questionably low, but K_{iB} (3.4 μM) for case BC, though high, is within the range of values reported.

The agreement between the model and data seen in Fig. 4 for cases AA and AB, where the K_is are optimized as a single value or independently, shows that acceptable agreement can be obtained without enhanced release. However, when release is included, as in cases ABC and AAC, slightly better agreement with the data is obtained. The values of the inhibition constants when optimized to be identical (1.7 μM for both constants in case AA; 1.8 μM for both constants in case AAC) are similar whether release is included or not. These values also fall between those obtained for the independently optimized inhibition constants in corresponding cases without and with release (K_{iA}=1.5 μM and K_{iB}=1.8 μM in case AB; K_{iA}=1.2 μM and K_{iB}=4.2 μM for case ABC). The inhibition constants generated by the optimization process for all of these cases are within the range published values.

Optimizing the two K_is independently does improve the agreement between the model and the data. Cases ABC and AB have somewhat lower errors (25.8 and 28.7) than their more restricted counterparts, cases AAC and AA (28.1 and 28.9), but this difference is insignificant. It is conceivable that since these two uptake processes are different kinetically (28), they might have different inhibition constants. However, the results presented here show that different constants are not required to model the response of extracellular DA under these conditions. In fact, the K_is determined by the optimization process for cases AAC and AA are very near the 1.5 μM mean of reported values. A simpler more restricted model, consisting of identical K_is, either with or without release, is able to give an extracellular DA response to cocaine very similar to that observed.

The enhanced release parameter generated by the optimization processes for cases AAC and ABC is 0.57 and 3.0 times the cocaine concentration, respectively, giving a maximum effect (coinciding with the maximum cocaine concentration) of 6 and 30 times basal release rates. The optimization of these two cases predicts that substantially more release is required to model the data when the pre- and postsynaptic uptake processes are inhibited differently (K_i values different) than when both processes are inhibited the same (K_i values the same). With a K_i value of 4.21 μM (ABC) for postsynaptic uptake, inhibition is less than with a K_i of 1.82 μM (AAC), thus more release is required to increase the extracellular DA concentration.

The processes of DA metabolism are included in the model, so modeled DOPAC and HVA responses to cocaine are possible. Total DA, and the DA metabolites DOPAC and HVA following cocaine administration for the cases in Fig. 4 are shown in Fig. 5. For the synthesis only effect, S, the model predicts that DOPAC, HVA, and 3-MT would decline only slightly, and total DA would decline to 85% of steady state levels. Only case ABC, having significantly enhanced release (30 times the basal release rate at maximum), is significantly different from the other cases for DOPAC, HVA, and total DA. The model predicts that a 40% increase in DOPAC over basal levels should be observed for this case. Additionally, HVA is predicted to increase to 30% of control. Total DA is predicted to decrease to 60% of basal levels. The 3 other cases shown in Fig. 5 (AA, AB, and AAC) do not significantly differ from each other or from the synthesis only effect. For all 4 cases (AA, AB, AAC, and ABC), modeled 3-MT increases to 200% of basal levels (data not shown), closely tracing the time course of the cocaine and extracellular DA curves.

DISCUSSION

The combination of data on extracellular DA and ex-

tracellular cocaine with mathematical modeling techniques gives insight into the cocaine uptake inhibition/release issue. It allows examination of the various combinations of the cocaine uptake inhibition and releasing effects on the dopaminergic nerve terminal. The extracellular DA concentration profile observed following a 30 mg/kg IP cocaine injection can be modeled if both pre- and postsynaptic uptake are competitively inhibited by cocaine with or without enhanced DA release. Agreement between data on extracellular DA and modeled extracellular DA is obtained whether or not a releasing effect is included. However, the metabolic consequences of these different possibilities are quite different, as discussed later.

Effect of Value of K_i

A value for K_i corresponding to the mean of reported values for K_i of 1.5 μM produces only a small decrease in the rate of presynaptic uptake, as seen in Fig. 3. The postsynaptic uptake rate, using a K_m of 15.8 μM (data not shown), is similarly affected by a K_i of 1.5 μM. Patrick *et al.* have reported a 41% reduction relative to control of DA uptake for 10 μM cocaine (35). A cocaine concentration of 10 μM corresponds to the maximum extracellular cocaine concentration observed for a 30 mg/kg IP dose (32). This reduction in uptake rate would correspond to a K_i value of about 0.5 μM. From Fig. 3 it can be concluded that the value of K_i has a considerable effect on the extent of inhibition of presynaptic uptake. Therefore, any conclusions drawn about cocaine's effects on DA uptake must take the value of K_i into account.

The results presented here show that different uptake inhibition constants for the two uptake processes are not required to model the response of extracellular DA to cocaine in the striatum. Also, the K_is obtained for cases AAC (both constants 1.66 μM) and AA (both constants 1.82 μM) are very near the 1.5 μM mean of reported values. A model consisting of identical K_is for both uptake processes, either with or without release, is able to give an extracellular DA response to cocaine in agreement with that observed.

It is interesting to note that very little change in steady state pre- or postsynaptic uptake rate is observed for a K_i of 1.5 μM (see Fig. 3). Little change in the uptake rate does not imply that cocaine inhibition of DA uptake is not significant. Rather, it means that the extracellular concentration changes of both cocaine and DA following a 30 mg/kg IP dose, when taken together, result in little effect on the presynaptic uptake rate. It is important to realize that the rate of uptake is dependent both on the concentration of DA and on the concentration of cocaine. Increased cocaine decreases the uptake rate, which increases extracellular DA. This increase in extracellular DA increases the uptake rate to value near the steady state rate of uptake. The concepts of uptake inhibition and uptake rate are related, but are not synonymous.

Metabolic Consequences

From the results of Fig. 4 alone, the neurochemical action of cocaine on the dopaminergic nerve terminal is not clear since both cases with and without enhanced DA release give similar results. It is clear, however, that the single processes of either pre- or postsynaptic uptake inhibition, or release, alone, will not produce extracellular DA responses in agreement with those observed.

Further information is required to distinguish between the cases shown in Fig. 4 since similar error is observed for

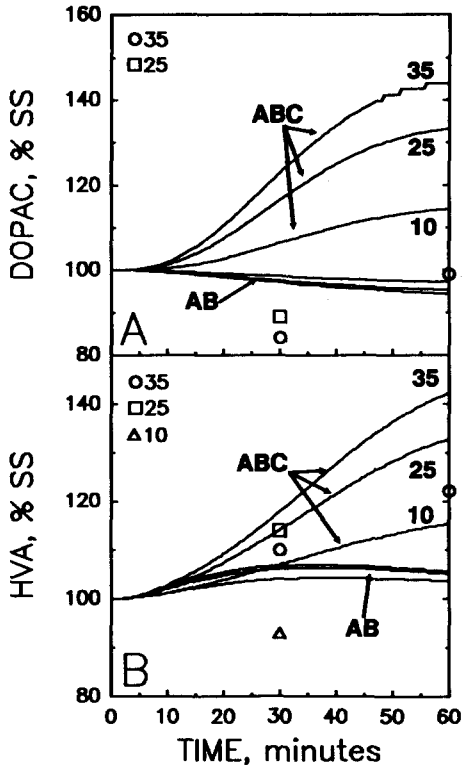

FIG. 6. Comparison of previously published and modeled DOPAC and HVA responses to different doses of IP cocaine. The symbols in Fig. 6 correspond to literature values, expressed as a percent of control. The numbers refer to the cocaine dose in mg/kg. The solid lines are modeled responses (percent of steady state values), for cases AB and ABC, for the cocaine doses (10, 25, and 35 mg/kg IP) used in the references. The circles correspond to the data for a 35 mg/kg (46), the squares for a 25 mg/kg dose (46), and the triangle for a 10 mg/kg (49).

cases with and without enhanced release. Levels of total DA, and the DA metabolites DOPAC and HVA following cocaine administration provide further information to address the difference, if any, between the combinations of uptake inhibition and releasing effects. The effects of cocaine on DOPAC, HVA, total DA, and 3-MT have been reported by others (14, 46, 49). The processes of DA metabolism are included in the model, so comparisons with modeled DOPAC and HVA responses to cocaine are possible.

Figure 5 shows the model predicted responses for the experimentally observable DA metabolites DOPAC and HVA, and for total DA, corresponding to the optimized cases shown in Fig. 4. Modeled 3-MT is almost identical for all 4 cases (AA, AB, AAC, and ABC), due to the fact it closely follows extracellular DA. Therefore, 3-MT provides no help in the discrimination between the inhibition and release effects of cocaine in the model. Total DA decreases in all cases. It is thus not practical to distinguish between cocaine effects based on total DA. DOPAC and HVA are different in case ABC from the other cases. DOPAC and HVA for the three other cases, AA, AB, and AAC, do not differ from each other or from the synthesis only effect. If cocaine causes significantly enhanced release, as in case ABC, the model predicts that DOPAC should show a marked increase. However, if there is little or no enhanced release effect of

cocaine, there should be little or no change in the DOPAC levels. Therefore, it should be possible to differentiate uptake inhibition and release effects on the basis of the change in DOPAC following cocaine administration. HVA should also provide the same information, however, the effect is not as pronounced.

A comparison of previously reported data with modeled DOPAC and HVA is presented in Fig. 6. The symbols in Fig. 6 correspond to literature values expressed as a percent of control. The solid lines are modeled responses for cases AB and ABC for doses (10, 25 and 35 mg/kg IP) used in the references. Since maximum extracellular DA and extracellular cocaine concentrations are linear with respect to IP cocaine dose (32), the cocaine response (Eqn. 1) for other doses can be modeled with these methods by adjusting the magnitude of the cocaine concentration profile. DOPAC responses are shown in Fig. 6A, and HVA in Fig. 6B.

Scheel-Kruger *et al.* (Fig. 6, circles) report HVA concentrations at 110% of control and DOPAC at 84% of control at 30 minutes for a 35 mg/kg IP cocaine injection (46). They also report an increase in HVA to 122% of control and DOPAC returns to control levels at 1 hour after the same dose. For a 25 mg/kg dose (Fig. 6, squares), they reported a small increase in HVA (114% of control) and a slight decline in DOPAC (89%) at 30 minutes. Taylor *et al.* (Fig. 6, triangles) observed a small decrease in HVA (93% of control) 30 min after a 10 mg/kg IP dose of cocaine (49). Flint *et al.* also reported little change in DOPAC from striatal slice experiments for 10 μM cocaine (14). This concentration of cocaine corresponds to the observed in vivo maximum concentration for a 30 mg/kg IP dose (32).

The modeled DOPAC data only shows an increase over steady state if there is enhanced release. Model responses for HVA, as seen in Fig. 6B, exhibit either a slight increase for no release (case AB), or a significant increase for release (case ABC). All of these investigator's metabolite results agree with the no release case (AB) in Figs. 6A and B. They do not agree with case ABC, the case including enhanced DA release.

In summary, although the extracellular DA concentration observed following a 30 mg/kg IP cocaine injection can be modeled with both pre- and postsynaptic uptake inhibition and with or without enhanced DA release, limited metabolite data from the literature for similar cocaine doses agrees best with modeled metabolite predictions for cases with no enhanced DA release. The DA uptake inhibition constants, K_{iA} and K_{iB}, obtained by the simplex optimization of the dopaminergic nerve terminal model for these cases agree well with values measured by others.

The combination of mathematical modeling with experimental extracellular DA, DOPAC, and HVA data, allows distinction between the release and uptake inhibition effects of cocaine on the striatal dopaminergic system, providing an useful tool in deciphering neurochemical drug effects. These methods could be used to examine the neurochemical effects of other drugs with multiple effects on DA, such as amphetamine.

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