Biochemical Pharmacology, Vol. 35, No. 22, pp. 4065–4071, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00
Pergamon Journals Ltd.

A KINETIC STUDY OF THE *IN VITRO* UPTAKE OF [^{3}H]DOPAMINE OVER A WIDE RANGE OF CONCENTRATIONS BY RAT STRIATAL PREPARATIONS

STEWART E. MIREYLEES,* NEIL T. BRAMMER† and GEORGE A. BUCKLEY
Department of Life Sciences, Trent Polytechnic, Nottingham NG11 2NS, U.K.

(*Received* 24 *February* 1986; *accepted* 17 *June* 1986)

Abstract—The uptake of [^{3}H]dopamine was investigated in a number of rat striatal preparations. Kinetic analysis of the rate of uptake by striatal slices indicated that at least two different saturable mechanisms for the catecholamine exist in this region, in addition to a first-order component. One of the uptake mechanisms has a high affinity for [^{3}H]dopamine and the other a low affinity. Distinct high and low affinity systems were also observed for the uptake of [^{3}H]dopamine by dispersed cell preparations of the rat striatum. Striatal synaptosomes appeared to have only a high affinity mechanism and a first-order component. The high affinity mechanisms are active transport mechanisms but the low affinity mechanisms are not unequivocably active transport systems. It is concluded that at least two sites for the uptake of [^{3}H]dopamine exist in the striatum and that the low affinity system is unlikely to be found at the presynaptic nerve ending and is not due to the presence of blood vessels.

In vitro studies have shown that, in a variety of innervated tissues, radiolabelled putative neurotransmitters are transported from the incubation medium into the cells [1–6]. Kinetic studies have shown that in peripheral tissues two distinct mechanisms can be described for noradrenaline, Uptake$_1$ and Uptake$_2$ [1, 7]. Uptake$_1$ has been identified as a neuronal system [7, 8] with the kinetic characteristics of high affinity and low capacity [1, 7]. While Uptake$_2$, which has a low affinity and high capacity for noradrenaline transport [7], is located extraneuronally [5, 9].

Studies with brain preparations have shown that uptake of the monoamines—dopamine, noradrenaline and 5-hydroxytryptamine—is mediated by respective high affinity active transport mechanisms [3, 4, 6, 10–15]. In addition, each amine could be taken up by a low affinity system [3, 4]. The high affinity mechanism for noradrenaline uptake in brain is similar to peripheral Uptake$_1$ [14, 16]. But the low affinity mechanisms, however, have kinetic constants which are not similar to those of peripheral Uptake$_2$ and the evidence suggests that these mechanisms are in fact neuronal but on the membrane of a different aminergic fibre [4]. Evidence for the existence of a central low affinity system, resembling peripheral Uptake$_2$, was found when rat brain slices were incubated in [^{3}H]normetanephrine [17].

In vivo studies, using cyclic voltammetry to monitor the clearance of released dopamine from extracellular fluid, suggest that dopamine is removed by several mechanisms [18] including a high capacity system which is distinct from high affinity neuronal uptake [19, 20]. We have already presented the results of a preliminary *in vitro* study which show the existence of a low affinity, high capacity system in striatal slices [21]. The objective of this present study was to investigate the low affinity, high capacity site further, in particular to test its specificity for substrate binding, energy requirement, and location. This involved incubating striatal preparations in a wide range of [^{3}H]dopamine concentrations for short periods. An allowance for [^{3}H]dopamine in the extracellular compartment was determined by the concurrent use of an extracellular marker. Results were analysed using a computer curve-fitting technique to compare the experimental results with three models of uptake.

MATERIALS AND METHODS

Materials. [7,8-^{3}H]Dopamine (46 Ci/mmol) and d-[U-^{14}C]sorbitol (302 mCi/mmol) were purchased from Amersham International plc, dopamine hydrochloride, L-noradrenaline bitartrate, L-adrenaline bitartrate, tyramine hydrochloride, β-phenylethylamine hydrochloride and ouabain octahydrate were purchased from Sigma Chemical Co. Ltd., Poole, U.K. Fisofluor was purchased from Fisons plc, Loughborough, U.K. Soluene-350 was purchased from Packard Instrument Co. All other chemicals were purchased from BDH Chemicals, Poole, U.K.

Preparation and incubation of slices. In all studies male Sprague–Dawley Rats weighing 175–250 g were used. The rats were stunned, decapitated, the brain removed and chilled in ice-cold Krebs' buffer (composition (mM): NaCl, 118; KCl, 4.75; KH_2PO_4, 1.19; $CaCl_2 \cdot 6H_2O$, 2.55; $MgSO_4 \cdot 7H_2O$, 1.2; $NaHCO_3$, 25; glucose 5.56; ascorbic acid, 0.2; pH 7.4 and bubbled with 5% CO_2 in oxygen). The corpora stri-

* To whom all correspondence should be addressed.
† Present address: Wyeth Laboratories, Taplow, Maidenhead, Berkshire SL6 0PH, U.K.

ata were dissected as described by Glowinski and Iversen [22]. The striata were chopped at right angles to the long axis into 0.5-mm-thick slices using a McIlwain tissue chopper. Slices prepared from three rats were pooled and randomised so that enough tissue was available to incubate simultaneously at all the concentrations of substrate. Six slices, which provided about 15 mg of tissue, were placed in each incubation flask which contained 9 ml of ice-cold oxygenated Krebs' buffer. After preincubating the tissue at 37° with aeration for 5 min, 1 ml of a freshly prepared stock solution containing [^{14}C]sorbitol and [^{3}H]dopamine was added. This gave an incubation solution with a [^{14}C]sorbitol concentration of 100 nCi/ml and [^{3}H]dopamine concentration of 100 nCi/ml: 0.1–500 μM. The concentration of the dopamine was adjusted by adding unlabelled dopamine to the stock solution. Incubation was continued for 2 min at 37° with aeration. This incubation period was chosen as it had been found that over the range of concentrations used uptake was temporally linear. Incubation was terminated by tipping the contents of the flask into a nylon mesh, trapping the slices which were rinsed quickly with 10 ml of ice-cold preincubation solution and blotted. The slices from each flask were placed in pre-weighed scintillation vials and the vials reweighed to obtain the weight of the tissue. The slices were digested with 0.5 ml Soluene-350, then neutralised by addition of 0.5 ml 1 M HCl before adding 10 ml Fisofluor-3 scintilation fluid. A 100 μl sample of each incubation medium was also taken to measure radioactivity. Samples were counted in a Packard Tri-carb 300C liquid scintillation counter. After quench correction, tissue [^{14}C]sorbitol content was expressed as the tissue/medium ratio (ml/g) and was taken to be a measure of the size of the extracellular compartment. Tissue tritium content was corrected for tritium in the extracellular space and was taken as a measure of dopamine uptake, which was expressed as nmol/g wet wt/min. Each experiment was repeated at least six times.

Preparation and incubation of dispersed cells. The required area of tissue was dissected as described above. The tissue was chopped into 0.1-mm cubes using a McIlwain tissue chopper and dispersed cells prepared by the method of Norton and Poduslo [23]. This involved incubating the cubes of tissue from the two corpora striata of each rat in 5 ml of hypertonic, hexose acid buffer medium containing 5% glucose, 5% fructose, 0.1 M phosphate buffer (pH 6), 1% bovine serum albumin and 1% trypsin. After incubating for 5 min at 37°, 5 ml of ice-cold oxygenated Krebs' buffer was added. The softened tissue was disrupted by passing it through a series of nylon meshes with pore sizes from 200 to 60 μm under a slight vacuum. The resultant cell suspension was centrifuged at 2000 *g* for 2 min. The supernatant liquid was discarded and the pellet resuspended in 1 ml of ice-cold Krebs' buffer. This results in a cell suspension containing neural soma, axon fragments and glial cells but not astrocytes [23]. A 200 μl aliquot of the cell suspension was added to 1.6 ml of oxygenated Krebs' buffer and preincubated for 5 min at 37°. Following the preincubation, 200 μl of a stock solution containing [^{14}C]sorbitol and [^{3}H]dopamine was added to give a dopamine concentration in the range 0.1–500 μM. Incubation was continued for 5 min. At the end of this period 1 ml of the incubation mixture was passed through a nitrocellulose filter (pore size 8.0 μm) under vacuum. Trial experiments had shown that this pore size was large enough to retain cells but not small fragments such as terminals (synaptosomes). The filter membrane was washed quickly by passing 2 ml ice-cold Krebs' buffer through it and placed in a scintillation counting vial and 10 ml of scintillation fluid (Fisofluor-3) added. The filter dissolved in the scintillant after about 3 hr and was ready for counting. A 500 μl sample of incubated cell suspension was assayed for protein [24]. It was found that [^{3}H]dopamine and [^{14}C]sorbitol will bind to the nitrocellulose filters in the absence of tissue but the ratio of [^{3}H]:[^{14}C] was found to be consistent for each concentration of dopamine. [^{14}C]sorbitol was, therefore, included in the incubation medium so that non-tissue bound tritium could be calculated. When tissue was present the filter tritium content was corrected for non-tissue tritium and was taken as a measure of dopamine uptake, which was expressed in terms of nmol/g prot/min.

Preparation and incubation of synaptosomes. The required area of tissue was dissected from a rat as described above and homogenised in 9 vols of 0.32 M sucrose solution containing 10 mM Tris–HCl (pH 7.4). The homogenate was centrifuged at 900 *g* for 10 min and the supernatant recentrifuged under the same conditions. The resultant supernatant was centrifuged at 10,000 *g* for 20 min. The pellet, containing a crude synaptosomal fraction, was resuspended and placed on the top of a Ficoll discontinuous density gradient (13% and 7.5%) and centrifuged at 55,000 *g* for 45 min. The 13%/7.5% interphase contained synaptosomes [26]. This band was resuspended in 1 ml of ice-cold Krebs' buffer. A 200 μl aliquot of the synaptosomal preparation was added to 1.6 ml of oxygenated Krebs' buffer and incubation and measurement of rate of dopamine uptake was as for dispersed cells except that the synaptosomes were collected on nitrocellulose filters with a pore size of 0.45 μm.

Incubation in the presence of test substances, low sodium medium or at 0°. When a drug was being tested for an inhibitory effect it was included in the preincubation medium and in the stock solution containing the radiolabelled substances. Controls, containing no test drug, were incubated at the same time.

In some experiments tissue was incubated in a modified Krebs' buffer which was isotonic but had a reduced sodium content (25 mM). Two low sodium media were used: one medium contained choline chloride (118 mM) [low sodium (choline Cl)] to maintain tonicity and the other contained sucrose (220 mM) [low sodium (sucrose)].

In experiments performed at 0° the incubation flasks were kept on ice throughout the duration of the experiment rather than transferring to a bath at 37° for preincubation and incubation. Controls at 37° were performed at the same time as those at 0°.

Kinetic analysis of uptake data. The results were analysed using an iterative least-squares curve-fitting

computer program to compare the results with three different models of uptake: (1) one saturable system (Michelis–Menten kinetics) plus first-order kinetics; (2) two saturable systems; and (3) two saturable systems and first-order kinetics. At each point the difference between the experimental value and the model value was divided by the respective standard error to determine the deviation in units of 'standard errors'. The overall fit of the model to the experimental results was based on the largest deviation at the individual points. If the largest deviation was greater than 1 standard error (>1 SE), the fit was considered as not good.

RESULTS

Uptake of [^{3}H]*dopamine into striatal slices*

The rate of uptake of [^{3}H]dopamine into striatal slices was investigated for dopamine concentrations in the range 0.1–500 μM. When the uptake data are plotted as graphs of substrate concentration/uptake velocity against substrate concentration (S/V vs S graphs) the points do not fall on a straight line but form a curve as shown in Fig. 1. This indicates that the uptake kinetics are not simply first-order kinetics nor those of a single saturable uptake mechanism. The shape of the curve suggests that a combination of saturable uptake mechanisms and first order kinetics may be present. Kinetic analysis using models (1) and (2) showed that the experimental data were not a good fit to these models and it is considered that these models are inappropriate. Using model (3) the analysis produced a very good fit; no experimental value is greater than 1/10th of a standard error different from the value determined by the model, i.e. the data fit to <1/10 SE.

The analysis gave a value of 0.095 μM for K_m and 0.096 nmol/g/min for V_{max} of one saturable uptake system, 40.7 μM for K_m and 22.4 nmol/g/min for V_{max} of the second uptake system and 0.145/min for the rate constant of the first-order component.

Uptake of [^{3}H]*dopamine into dispersed striatal cells*

The uptake of [^{3}H]dopamine over a concentration range of 0.1–500 μM by dispersed striatal cells produced a graph which was similar in shape to that obtained with striatal slices (Fig. 2). Curve-fitting analysis failed to produce a good fit with models (1) and (2). Model (3) produced a good fit which was to <1/10 SE. The analysis gave a value of 0.033 μM for K_m and 5.73 nmol/g prot/min for V_{max} of one saturable system, 3.06 μM for K_m and 31.2 nmol/g prot/min for V_{max} of the second uptake system and 2.67/min for the rate constant of the first-order component.

Uptake of [^{3}H]*dopamine into striatal synaptosomes*

A kinetic plot of the uptake of [^{3}H]dopamine into striatal synaptosomes produced a curve (Fig. 3) similar to those found for the slices and dispersed striatal cells. However, although the general shape of this curve was similar to the other two, the curve-fitting analyses produced different results. The computer could fit the experimental data to all three models with a fit to <1/2 SE. The results for the low affinity mechanisms in models (2) and (3) produced

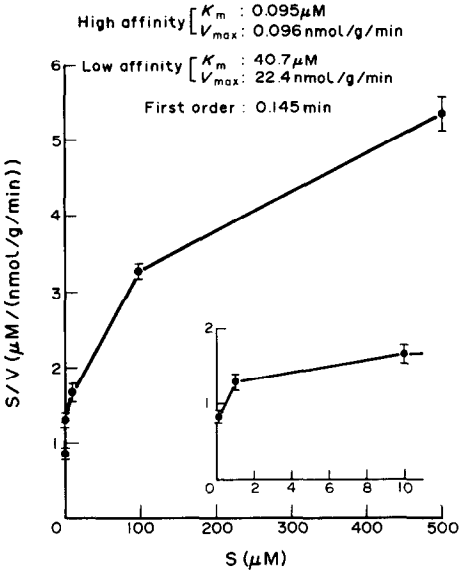

Fig. 1. Kinetics of uptake of [^{3}H]dopamine by rat striatal slices. Rat striatal slices were incubated for 2 min in the presence of [^{3}H]dopamine at a concentration in the range 0.1–500 μM. Results for the velocity of uptake (V) (nmol/g/min) and concentration of substrate (S) (μM) were plotted as the S/V vs S transformation of the Michelis–Menten equation. Each point is the mean ± SEM of at least 36 determinations. The inset shows an expansion of the results for the concentration range 0.1–10 μM. Kinetic analysis was by computer using a curve-fitting program incorporating a model of two saturable systems and a first-order system. Results of the kinetic analysis are shown.

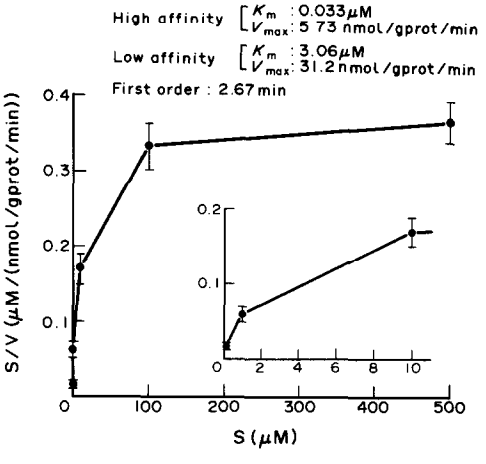

Fig. 2. Kinetics of uptake of [^{3}H]dopamine by dispersed striatal cells. Rat striatal cells were incubated for 5 min in the presence of [^{3}H]dopamine at a concentration in the range 0.1–500 μM. Results for the velocity of uptake (V) (nmol/g prot/min) and concentration of substrate (S) (μM) were plotted as the S/V vs S transformation of the Michelis–Menten equation. Each point is the mean ± SEM of at least nine determinations. The inset shows an expansion of the results for the concentration range 0.1–10 μM. Kinetic analysis was by computer using a curve fitting program incorporating a model of two saturable systems and a first-order system. Results of the kinetic analysis are shown.

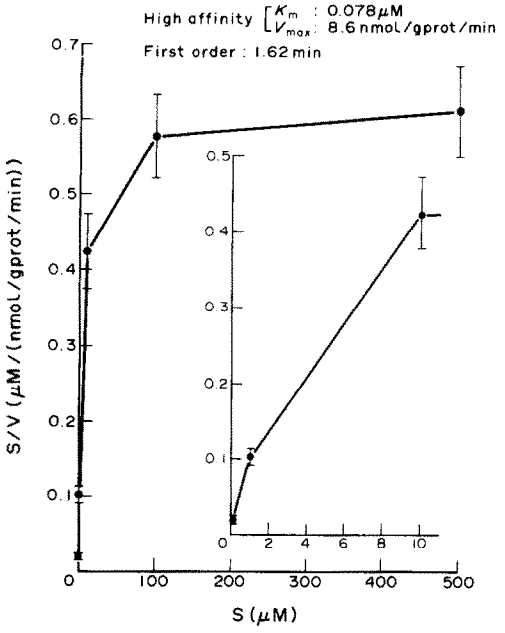

Fig. 3. Kinetics of uptake of [^{3}H]dopamine by rat striatal synaptosomes. Rat striatal synaptosomes were incubated for 5 min in the presence of [^{3}H]dopamine at a concentration in the range 0.1–500 μM. Results for the velocity of uptake (V) (nmol/g prot/min) and concentration of substrate (S) (μM) were plotted as the S/V vs S transformation of the Michelis–Menten equation. Each point is the mean ± SEM of at least six determinations. The inset shows an expansion of the results for the concentration range 0.1–10 μM. Kinetic analysis was by computer using a curve fitting program incorporating a model of one saturable system and a first-order system. Results of the kinetic analysis are shown.

values for K_m in the range 9×10^6–6×10^8 μM with similarly high values for V_{max}. It was concluded from these values that the low affinity mechanisms obtained with models (2) and (3) were representations of a first-order component and it is considered that model (1) was the most compatible of the three with the experimental data. The results obtained were 0.078 μM for K_m and 8.6 nmol/g prot/min for V_{max} for the saturable uptake system and 1.62/min for the rate constant of the first-order component.

Contribution of each uptake system to uptake in slices and dispersed cells

The contributions of each component of model (3) to total uptake at each substrate concentration were calculated using the values for K_m and V_{max} which had been derived from kinetic analysis of the data from slices and dispersed cells. These results are summarised in Table 1.

Uptake of [^{3}H]dopamine into slices in the presence of test substances

The uptake of [^{3}H]dopamine from a medium containing 100 μM dopamine was measured in the presence of various test substances which were at a concentration of 10^{-3} M. The results are summarised in Table 2. Metanephrine, methylamine, catechol and β-phenylethylamine had no significant effect on [^{3}H]dopamine uptake. But adrenaline, noradrenaline, tyramine and dopamine significantly inhibited [^{3}H]dopamine uptake. At 100 μM [^{3}H]dopamine the low affinity mechanism will account for 52% of the total uptake and the high affinity system for less than 1%. Dopamine produced the greatest inhibition, 55.15 ± 5.1%; a value which is not significantly different from the contribution of the saturable systems to total uptake in slices.

Table 1. Percent contributions of the various uptake systems to total uptake of [^{3}H]dopamine in rat striatal slices and dispersed cells

Uptake system	[^{3}H]dopamine concentration (μM) 0.1	1.0	10	100	500
			Striatal slices		
High affinity	41.5	11.4	1.6	(0.003)	(0.001)
Low affinity	46.3	69.8	74.1	52.2	22.2
First order	12.2	18.8	24.3	47.5	77.5
			Dispersed cells		
High affinity	77.5	34.9	10.1	1.9	(0.004)
Low affinity	17.7	48.3	42.4	10.0	2.3
First order	4.8	16.8	47.4	88.1	97.3

The value for each system was calculated from the respective kinetic constants which had been obtained from kinetic analysis using model (3) (two saturable uptake mechanisms showing Michelis–Menten kinetics and a first-order system). Total uptake was the sum of the three components.

Kinetic constants. Slices: high affinity: $K_m = 0.095$ μM; $V_{max} = 0.096$ nmol/g/min; low affinity: $K_m = 40.7$ μM, $V_{max} = 22.4$ nmol/g/min; first order = 0.145/min.

Dispersed cells: high affinity: $K_m = 0.033$ μM, $V_{max} = 5.73$ nmol/g prot/min; low affinity: $K_m = 3.06$ μM, $V_{max} = 31.2$ nmol/g prot/min; first order = 2.67/min.

Table 2. Effect of test substances on the uptake of [^{3}H]dopamine by striatal slices

Substance	% Inhibition
Catechol	0.19 ± 2.4
Methylamine	6.09 ± 2.4
β-Phenylethylamine	9.89 ± 3.9
Tyramine	35.64 ± 2.7*
Dopamine	55.15 ± 5.1*
Noradrenaline	42.94 ± 5.8*
Adrenaline	39.16 ± 7.4*
Metanephrine	−5.86 ± 4.7

Rat striatal slices were preincubated for 5 min in Krebs' buffer containing the test drug at a concentration of 10^{-3} M. [^{3}H]Dopamine was added to give a final concentration of 100 μM and incubation continued for 2 min. Each result, expressed as percent inhibition of control uptake, is the mean ± SEM of at least six determinations. Those marked * are significantly different from control ($P < 0.05$).

Control uptake was 30.44 ± 1.6 nmol/g/min.

Table 3. Percent inhibition of the uptake of [^{3}H]dopamine into striatal slices or dispersed cells produced by low sodium medium, dinitrophenol, ouabain or incubation at 0°

Condition	Striatal slices [^{3}H]Dopamine conc. 0.1 μM	Striatal slices [^{3}H]Dopamine conc. 100 μM	Striatal cells [^{3}H]Dopamine conc. 0.1 μM	Striatal cells [^{3}H]Dopamine conc. 10 μM
Low-Na (sucrose)	29.5 ± 10.7*	8.9 ± 4.4	10.5 ± 8.3	6.5 ± 6.7
Low-Na (choline Cl)	82.3 ± 1.3*	40.0 ± 2.2*	66.3 ± 4.4*	52.0 ± 12.2*
Ouabain (10^{-3} M)	79.5 ± 3.0*	40.0 ± 4.0*		
Dinitrophenol (10^{-3} M)	81.3 ± 5.0*	58.1 ± 5.4*	67.8 ± 3.3*	70.8 ± 6.0*
0° incubation	83.5 ± 4.1*	85.0 ± 2.5*	96.0 ± 0.21*	95.0 ± 0.52*

Slices or dispersed cells of rat striatum were preincubated for 5 min in modified Krebs' buffer. In studies with slices [^{3}H]dopamine was added to give a final concentration of 0.1 and 100 μM and incubation continued for 2 min. In studies with dispersed cells [^{3}H]dopamine was added to give final concentrations of 0.1 and 10 μM and incubation continued for 5 min. In experiments at 0° unmodified Krebs' buffer was used and preincubation and incubation were at 0°. Each result, expressed as percent inhibition of control uptake, is the mean ± SEM of at least six determinations. Those marked * are significantly different from control ($P < 0.05$).

Control uptake in slices: 0.1 μM, 0.151 ± 0.008 nmol/g/min; 100 μM, 29.9 ± 1.57 nmol/g/min. Control uptake in dispersed cells: 0.1 μM, 5.56 ± 0.62 nmol/g prot/min; 10 μM, 58.8 ± 6.57 nmol/g prot/min.

Uptake in the presence of dinitrophenol, ouabain, low sodium or at 0°

The results for the uptake of [^{3}H]dopamine into striatal slices and dispersed cells when incubated in low sodium medium, in the presence of dinitrophenol or ouabain or at 0° are summarised in Table 3. Low sodium, dinitrophenol, ouabain and 0° significantly inhibited the uptake of 0.1 μM [^{3}H]dopamine into slices. Most of these treatments produced about 80% inhibition; a value which is similar to that obtained for the sum of the contributions of the two carrier mediated systems in slices (Table 1). When [^{3}H]dopamine was present at a concentration of 100 μM, uptake by slices was significantly inhibited by all conditions except the low sodium (sucrose) medium. At this concentration of [^{3}H]dopamine the low affinity system accounts for 52.2% of the uptake, while the high affinity system contributes less than 1% (Table 1). The levels of inhibition of uptake by either low sodium (choline Cl) medium, or the presence of ouabain or dinitrophenol are below or not significantly different from this value. But the inhibition produced by incubating at 0° greatly exceeds this value.

When striatal cells were incubated in the same conditions as the slices, uptake of [^{3}H]dopamine at both 0.1 and 10 μM was significantly inhibited by all conditions except the low sodium (sucrose) medium. At 0.1 μM [^{3}H]dopamine, 95.2% of uptake in dispersed cells will be due to carrier systems (Table 1)—77.5% due to the high affinity system and 17.7% to the low affinity system. Only incubation at 0° produced a level of inhibition which is similar to the contribution of carrier mediated uptake. The other treatments produced lower levels of inhibition. At 10 μM [^{3}H]dopamine, low sodium (choline Cl) produced 52% inhibition of uptake, which is not significantly different from the 52.5% contribution of carrier mediated systems to uptake. However, incubation at 0° or with dinitrophenol produced a level of inhibition which is significantly higher than this.

Effect of test conditions on [^{14}C]sorbitol space in slices and dispersed cells

The size of the [^{14}C]sorbitol space in slices (0.309 ± 0.016 ml/g; N = 72) was unaffected by the concentration of dopamine. Generally the presence of the test substances had no effect on [^{14}C]sorbitol space. The main exception was the inclusion of dinitrophenol which caused a significant reduction of the [^{14}C]sorbitol space by 24.0 ± 3.9% (N = 12). Incubating at 0° also significantly reduced the sorbitol space by 46.4 ± 4.4% (N = 12).

Dispersed cells retained some [^{14}C]sorbitol at a tissue to medium ratio of 24.2 ± 1.02 ml/g protein (N = 24). This was reduced by 38.4 ± 5.2% (N = 12) by incubation at 0°.

DISCUSSION

An inherent problem of *in vitro* uptake experiments is that substrate is frequently trapped in the extracellular space as well as in the cellular compartment. Since it is usually the cellular compartment that is being studied, then allowances must be made for substrate in the extracellular space. The size of the extracellular space increases under *in vitro* conditions [26, 27] which makes it inappropriate to simply apply a constant correction factor. The methods used for compensating for substrate in the extracellular space are widely available in the literature. Commonly used ones are washing substrate from the extracellular space or incubating a set of preparations at 0° in parallel with those at 37°. These methods have disadvantages, namely, substrate which is not firmly bound in cells may be washed out or only temperature sensitive uptake will be measured, respectively. The two methods are satisfactory if specifically neuronal uptake is being investigated.

An alternative method is to incorporate an extracellular marker in the incubation medium. [^{14}C]Sorbitol was used in these experiments as it

has a molecular weight which is similar to that of [^{3}H]dopamine and hence should penetrate into the same extracellular spaces. Using an extracellular marker rather than the other methods makes no assumptions about the lability of accumulated substrate or temperature dependence of the uptake mechanisms.

S/V vs S graphs of the data for [^{3}H]dopamine uptake showed that a simple graphical or linear regression analysis of the data would not be possible. The graphs indicated that there was more than just a single saturable mechanism present. The results were analysed using a least-squares iterative curve-fitting program which contained an appropriate mathematical model for uptake. Three models were used. The simplest considered that there was one saturable system (that obeyed Michelis–Menten kinetics) and a system which obeyed first-order kinetics (unsaturable). The next model was based on two saturable systems. The third model had two saturable systems and a first-order component. The results for uptake into striatal slices were not a good fit to the two simpler models but they were a good fit to the third model. This suggests that the two simpler models were not appropriate descriptions of the results and were rejected. The fact that the results were a good fit to the third model does not in itself mean this model is a true description of the results. It simply indicates that the results and the model are compatible. A great deal of caution always has to be exercised when using computer model-fitting techniques. The more complicated the models that are used, the easier it is for a fit to be achieved. The kinetic parameters presented are from the simplest model which could produce a good fit. The high affinity uptake system in striatal slices found in this study has a K_m value of 0.095 μM and V_{max} value of 0.096 nmol/g/min. The K_m value is similar to those previously reported for the uptake of dopamine into dopamine nerve terminals [3, 4, 12, 28, 29]. The low affinity system described here has a K_m value of 40.7 μM and V_{max} value of 22.4 nmol/g/min. The value for V_{max} is comparable to the value obtained from *in vivo* studies [20]. The first-order component may correspond to passive diffusion or to a very low affinity site which appears as an apparent first-order component because of the model used in the curve-fitting analysis.

When tissue slices are used, substrate will be presented to the uptake sites in the centre of each slice after passing down a concentration gradient from the outside. This could result in the sites being presented with an appreciably lower concentration of substrate than that presented to the sites at the outside of the slice. Consequently, uptake sites in the centre could have an apparently lower affinity than those on the other parts of the slice, which would be reflected in the kinetic analysis having a low affinity site as an artifact.

If this were true, then preparations in which the cells of the tissue are dispersed should not show apparent low affinity sites since the substrate will be presented to all the cells simultaneously at the same concentration. Kinetic analysis of the uptake of [^{3}H]dopamine into dispersed striatal cells showed that both high and low affinity sites are present in the tissue which indicates that the low affinity sites found in slices were not artifacts. The high affinity site has a K_m value of 0.033 μM and V_{max} value of 5.73 nmol/g protein/min. The K_m is similar to the high affinity system found in slices. A direct comparison of V_{max} in the dispersed cell preparation with that in slices is not valid since the two preparations are not equivalent. The nitrocellulose filters, used to separate the dispersed cells from the incubation medium have a pore size of 8 μM and do not trap all the tissue fragments. They allow small fragments, such as separated axon terminals, to pass through. Also the method of preparation of the cells causes loss of astrocytes [23]. The dispersed cell preparation trapped on the filters will have a different cellular composition from that of slices. Another factor is that dispersing the cells may expose uptake sites to substrate which in slices may not otherwise be exposed. The K_m for the low affinity mechanism has a value of 3.06 μM and a V_{max} value of 31.2 nmol/g protein/min. The K_m value is much lower than that for the low affinity site found in slices.

The first-order component for uptake into the dispersed cells is much greater than in slices. This is probably a reflection of the increase in available surface area.

The uptake of [^{3}H]dopamine into synaptosomes showed different kinetic characteristics from those for uptake into slices or dispersed cells. Kinetic analysis could not provide evidence for low affinity sites. The K_m for the saturable uptake mechanism (0.078 μM) is similar to the K_m for the high affinity mechanisms found in the slices and the dispersed cells. V_{max} for the system in the synaptosomes (8.6 nmol/g protein/min) is higher than that in the dispersed cells. This may be a reflection of the material which was lost in the separation of the dispersed cells from the incubation medium, but which is not lost on filtering the synaptosomes. Lack of a low affinity mechanism in the synaptosomes suggests that the mechanism is not associated with nerve terminals. It is likely, therefore, that it is present on structures such as nerve cell bodies or non-neuronal cells.

In order to investigate the low affinity sites further, the effects of various substances on the uptake of [^{3}H]dopamine in slices were tested. To do this, a concentration of substrate was chosen (100 μM) in which the low affinity mechanism would be the predominant of the two saturable mechanisms. At this concentration neither methylamine, catechol nor β-phenylethylamine had any significant effect on [^{3}H]dopamine uptake in slices. Thus the low affinity site is not a non-selective site which simply binds with aromatic or cationic molecules. Tyramine, adrenaline, noradrenaline and dopamine showed an increasing ability to inhibit the uptake of [^{3}H]dopamine. But none of these compounds produced a level of inhibition which was significantly higher than the maximum contribution of the low affinity system to overall uptake. We have previously reported [21] that inhibition of uptake of [^{3}H]dopamine from a high concentration (100 μM) by slices shows a pharmacologically different pattern to that from a low concentration (0.1 μM)—indicating that the low and high affinity mechanisms

are dissimilar. Metanephrine, a potent inhibitor of peripheral Uptake$_2$ [7, 30] had no significant effect on the low affinity system in slices. Thus it is unlikely that the low affinity mechanism is due to uptake into vascular smooth muscle cells present in the slices.

It is generally accepted that the high affinity neuronal amine uptake systems are active transport mechanisms [13–15]. The uptake results for incubating striatal slices and dispersed cells, in conditions which inhibit active transport, are in accordance with this, particularly for incubations at 0.1 μM [^{3}H]dopamine. Complete inhibition of uptake has not been achieved but this is to be expected since the high affinity system is only one component of several involved in uptake. The levels of inhibition which have been achieved in the two preparations do not exceed the contributions due to carriers. But in slices the level is greater than would occur if the high affinity mechanism was the only active transport system present. This strongly suggests the low affinity system is active transport. It would, therefore, be expected that at a higher concentration of [^{3}H]dopamine, uptake would be inhibited to an extent dependent on the contribution of carrier-mediated transport to total uptake. In general, uptake from 100 μM [^{3}H]dopamine by slices and from 10 μM [^{3}H]dopamine by dispersed cells were inhibited by the various conditions, except the low sodium (sucrose) medium, to an extent which is similar to the contributions of carrier-mediated transport. The low inhibitory effect of the low sodium (sucrose) medium on the uptake of [^{3}H]dopamine cannot readily be explained but certainly weakens the evidence that the low affinity mechanisms are active transport.

In both slices and dispersed cells uptake of [^{3}H]dopamine at the higher concentration was inhibited by incubation at 0° to a greater level than the contribution of the two saturable mechanisms to total uptake; suggesting that the first-order component of uptake may be temperature sensitive. However, investigation of the effect of temperature on the sorbitol space showed that this compartment was temperature sensitive. The size of the compartment is 46.6% smaller at 0° than at 37°. Other workers [31] have shown a similar temperature sensitivity for extracellular markers, including sorbitol. They attributed the reduction in marker space to a system of temperature sensitive microtubules which connected to the extracellular space. This is supported by the observation that dispersed cells showed a temperature sensitive retention of [^{14}C]sorbitol. The large reduction in uptake of [^{3}H]dopamine due to incubation at 0° may in part be due to a reduction in the area of cell membrane accessible to [^{3}H]dopamine. The only other factor which influenced the size of the sorbitol space was the inclusion of dinitrophenol in the incubation medium. This caused a reduction of 24% in the size of the sorbitol space. But inhibition of [^{3}H]dopamine uptake was significantly greater than reduction in sorbitol space which suggests that uptake may be temperature and dinitrophenol sensitive.

In conclusion the results of this study show that in addition to a high affinity, low capacity uptake system there is a low affinity, high capacity mechanism for the uptake of [^{3}H]dopamine in the rat striatum. This mechanism may be present on neuronal cell bodies or in non-neuronal cells such as glia.

REFERENCES

1. L. L. Iversen, *Br. J. Pharmac.* **21**, 523 (1963).
2. B. Hamberger and D. Masuoka, *Acta pharmac. tox.* **22**, 363 (1965).
3. S. H. Snyder and J. T. Coyle, *J. Pharmac. exp. Ther.* **165**, 78 (1969).
4. E. G. Shaskan and S. H. Snyder, *J. Pharmac. exp. Ther.* **175**, 404 (1970).
5. O. V. Avakian and J. S. Gillespie, *Br. J. Pharmac.* **32**, 168 (1968).
6. S. B. Ross and A. L. Renyi, *Acta pharmac. tox.* **21**, 226 (1964).
7. L. L. Iversen, *Br. J. Pharmac.* **25**, 18 (1965).
8. G. Hertting, J. Axelrod, I. J. Kopin and L. G. Whitby, *Nature. Lond.* **189**, 66 (1961).
9. D. E. Clarke, C. J. Jones and P. A. Linley, *Br. J. Pharmac.* **15**, 707 (1969).
10. S. H. Snyder, A. I. Green and E. D. Hendley, *J. Pharmac. exp. Ther.* **164**, 90 (1968).
11. A. S. Horn, J. T. Coyle and S. H. Snyder, *Molec. Pharmac.* **7**, 66 (1971).
12. J. T. Coyle and S. H. Snyder, *J. Pharmac. exp. Ther.* **170**, 221 (1969).
13. L. L. Iversen, *Br. J. Pharmac.* **41**, 571 (1971).
14. L. L. Iversen, *Br. med. Bull.* **29**, 130 (1973).
15. M. J. Kuhar, *Life Sci.* **13**, 1623 (1973).
16. A. S. Horn, *Br. J. Pharmac.* **47**, 332 (1973).
17. E. D. Hendley, K. M. Taylor and S. H. Snyder, *Eur. J. Pharmac.* **12**, 167 (1970).
18. A. G. Ewing and R. M. Wightman, *J. Neurochem.* **43**, 570 (1984).
19. J. A. Stamford, Z. L. Kruk, J. Millar and R. M. Wightman, *Neurosci. Lett.* **51**, 133 (1984).
20. J. A. Stamford, Z. L. Kruk and J. Millar, *Brain Res.* **373**, 85 (1986).
21. N. T. Brammer, G. A. Buckley and S. E. Mireylees, *Br. J. Pharmac.* **81**, 112P (1984).
22. J. Glowinski and L. L. Iversen, *J. Neurochem.* **13**, 655 (1966).
23. W. T. Norton and S. E. Poduslo, *Science* **167**, 1144 (1970).
24. O. H. Lowry, N. J. Rosenbrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
25. C. W. Cotman and D. A. Matthews, *Biochim. biophys. Acta* **249**, 380 (1971).
26. J. R. Stern, L. V. Eggleston, R. Hems and H. A. Krebs, *Biochem. J.* **44**, 410 (1949).
27. H. S. Bachelard, W. J. Campbell and H. McIlwain, *Biochem. J.* **84**, 789 (1962).
28. J. E. Harris and R. J. Baldessarini, *Life Sci.* **13**, 303 (1973).
29. L. Tuomisto, J. Tuomisto and E. Smissman, *Eur. J. Pharmac.* **25**, 351 (1974).
30. A. S. V. Burgen and L. L. Iversen, *Br. J. Pharmac.* **25**, 34 (1965).
31. S. R. Cohen, P. F. Stampleman and A. Lajtha, *Brain Res.* **21**, 419 (1970).