Which Form of Dopamine Is the Substrate for the Human Dopamine Transporter: the Cationic or the Uncharged Species?*

(Received for publication, October 9, 1998, and in revised form, December 11, 1998)

Janet L. Berfield, Lijuan C. Wang, and Maarten E. A. Reith:

From the Department of Biomedical and Therapeutic Sciences, University of Illinois College of Medicine, Peoria, Illinois 61656

The question of which is the active form of dopamine for the neuronal dopamine transporter is addressed in HEK-293 cells expressing the human dopamine transporter. The $K_m$ value for $[^3H]dopamine$ uptake fell sharply when the pH was increased from 6.0 to 7.4 and then changed less between pH 7.4 and 8.2. The $K_m$ value for dopamine in inhibiting the cocaine analog $[^3H]2\beta$-carbomethoxy-3β-(4-fluorophenyl)tropane binding displayed an identical pH dependence, suggesting that changes in uptake result from changes in dopamine recognition. Dopamine can exist in the anionic, neutral, cationic, or zwitterionic form, and the contribution of each form was calculated. The contribution of the anionic form is extremely low (≤0.1%), and its pH dependence differs radically from that of dopamine binding. The increase in the neutral form upon raising the pH can model the results only when the $pK_a$ (equilibrium neutralcharged) is set to a much lower value (6.8) than reported for dopamine in solution (8.86). The sum of cationic and zwitterionic dopamine concentrations remained constant over the entire pH range studied. These forms are the likely transporter substrates with pH-dependent changes occurring in their interaction with the transporter. The binding of dopamine, a hydroxylated phe- nylethylamine derivative, displays the same pH dependence as guanethidine, a heptamethyleniminoethylguanidinium derivative fully protonated under our conditions. An ionizable residue in the transporter could be involved that does not interact with or impact the binding of bretylium, a quaternary ammonium phenylethylamine derivative that is always positively charged and shows only a minor reduction in $K_m$ upon increasing pH.

The dopamine ($DA$) transporter (DAT) in neuronal plasma membranes clears DA from the (extra)synaptic space (1–3) by an active uptake process with co-transport of Na$^+$ and Cl$^-$ (for recent reviews see Refs. 4 and 5) but probably not countertransport of K$^+$ (6). In calculating the overall stoichiometry of the neuronal DA uptake process as 2:1:1 for Na$^+$:Cl$^-$:DA, the authors have combined the evidence for co-transport of two Na$^+$ ions and one Cl$^-$ ion per DA molecule with the assumption that the cationic form of DA is the substrate for uptake (7–9). DA has an amino group that can accept a proton and a phenolic hydroxyl group that can donate a proton; the second phenolic group has a $pK_a$ value greater than 12. Therefore, except at extremely basic pH values where both hydroxyl groups can be dissociated, DA can exist as a cation ($H^+NDOH$, with D for DA skeleton), a zwitterion (H$^+NDO$), a neutral form (H$_2$NDOH), or an anion (H$_2$NDO$^-$). With $pK_a\beta$ and $pK_a\gamma$ values of 8.86 and 10.5, respectively (10–12) (see “Experimental Procedures”), it can be calculated that at physiological pH, DA exists mostly as a cation, which has prompted the assumption that this is the active form for transport (7–9). Indeed, in the analogous cases of neuronal uptake of serotonin and norepinephrine, strong evidence for translocation of the cationic form has been advanced (13, 14). Indirect evidence in favor of the cation also being the active form for DA uptake comes from site-directed mutagenesis studies (15) and molecular modeling (16) implicating an interaction between the protonated amine group of DA and Asp-79 of the DAT. The question of the active monoamine form for uptake by the vesicular monoamine transporter was given a great deal of attention more than a decade ago, with mixed conclusions. A case has been made for the cationic (17, 18) as well as the uncharged form (for review see Ref. 19), and to our knowledge no new information on this issue is available.

In the present study, the question of the active form of DA for the neuronal DAT is addressed by monitoring for the human DAT (hDAT) cloned by Janowsky and colleagues (20, 21) and the pH dependence of (i) uptake and binding of DA, which can be in an ionized or neutral form, and (ii) binding of guanethidine, which is positively charged in the pH range studied (see below), and bretylium, which is always positively charged as a quaternary ammonium salt. Uptake was measured by monitoring the accumulation of $[^3H]DA$, and binding was assessed indirectly through inhibition of high affinity binding of the cocaine analog $[^3H]2\beta$-carbomethoxy-3β-(4-fluorophenyl)tropane (WIN 35428), which interacts with a domain on the DAT that overlaps with the DA domain (5, 15, 16, 22–24). By comparing DA uptake and binding, we can determine whether changes in uptake as a function of pH are solely determined by changes at the level of DA recognition, the first step in the DA translocation cycle. In contrast to DA, which is a dihydroxy- lated phenylethylamine, bretylium is a nonhydroxylated bro mophenylmethylamine in which the amine is quaternary (25), and therefore the formation of an overall neutral species is not possible for this compound. Bretylium is usually employed for its inhibitory effect on norepinephrine release (see Ref. 26) and monoamine oxidase (27). In addition, it can be taken up into dopaminergic cells (28) and interferes with DA uptake into striatal synaptosomes (29). Guanethidine is a compound derived from guanidine. The latter is a strong base with a $pK_a$ of

---

* This work was supported by National Institute on Drug Abuse Grant DA 08379 (to M. E. A. R.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: Dept. of Biomedical and Therapeutic Sciences, University of Illinois College of Medicine, Box 1649, Peoria, IL 61656. Tel.: 309-671-8545; Fax: 309-671-8403; E-mail: MaartenR@uic.edu; Web: http://lnbd.uicomp.uic.edu/~maarten/maarten.htm.

The abbreviations and trivial names used are: DA, dopamine; DAT, DA transporter; hDAT, human DAT; ANOVA, analysis of variance; GBR 12935, 1-(2-diphenylmethoxy)-ethyl-4-(3-phenylpropyl)pipera zine; WIN 35428, 2β-carbomethoxy-3β-(4-fluorophenyl)tropane.
12.5 (30) and is therefore predominantly in the positive guanidine ion form over a wide pH range up to 11. Guanethidine has a $pK_a$ of 11.4 and a $pK_z$ of 8.3 (31). The former $pK_a$ value describes the ability of guanethidine, at the pH values between 6.0 and 8.2 studied here, to carry one proton at the guanidine residue, whereas the latter $pK_z$ value represents the ability of the guanido group to accept an additional proton as a function of pH (at pH 8.2 in ~50% of the molecules). In a similar but not identical manner as bretylium, guanethidine is known to interfere with catecholaminergic transmission in mammalian (32) and invertebrate (33) systems. Guanethidine can act as a substrate for the norepinephrine transporter (34) and has been reported to inhibit neuronal uptake of norepinephrine (25), but its affinity for the DAT, to our knowledge, has not been reported.

**EXPERIMENTAL PROCEDURES**

**Materials**—[3H]WIN 35428 (84.5 Ci/mmol) and [3H]dopamine (21.5 Ci/mmol) were from NEN Life Science Products. Unlabeled WIN 35428 was from the Research Triangle Institute (Research Triangle Park, NC). All other chemicals were from Sigma. Glass fiber filter mats and Betaplate Scint scintillation mixture for the binding assays were from Wallac Inc. (Gaithersburg, MD). GF/C fiber filter mats and CytoScint ES mixture for the uptake assays were from Brandel (Gaithersburg, MD) and ICN (Costa Mesa, CA), respectively. The source of the DAT was the HEK-293-hDAT cell line developed by Janowsky and co-workers (21).

[3H]WIN 35428 Binding Assay—The general conditions for growing the cells, working up the membrane preparations, and conducting the binding assays were as described previously by us (35). Briefly, after cell lysis, membranes were prepared and homogenized with the Brinkmann Polytron (setting 6, 15 s) in ice-cold saline. Binding assays were performed in ice-cold 30 mM sodium phosphate buffer (the result of mixing primary and half-strength secondary sodium phosphate buffer to a 1:1 ratio) between 60 °C and 80 °C (as indicated, or at room temperature) also containing 122 mM sodium chloride, 5 mM potassium chloride, 1.2 mM MgSO$_4$, 10 mM glucose, 1 mM CaCl$_2$, and 0.1 mM troponol for catechol-0-methyltransferase inhibition (21) (“assay buffer”). The listed pH values are those measured at room temperature after all of the above components had been added. In this buffer, binding assays were conducted for 10 min at 21 °C in 96-well plates with 4 nM [3H]WIN 35428. The assay mix contained the appropriate, nonradioactive WIN 35428 (1–300 nM), DA (0.5–100 μM), guanethidine (10–3,000 μM), or bretylium (10–3,000 μM) (for $K_a$ or $K_z$ estimation, respectively), HEK-293-hDAT membranes (~120 μg of protein) as determined by the method of Lowry; see Ref. 35) with or without 100 μM cocaine for assessment of nonspecific binding. Assays were terminated on glass fiber filter mats (presoaked in 0.05% (v/v) polyethyleneimine) with the MACH 3–96 Tomtec harvester (Wallac Inc.) using phosphate (10 mM)-buffered saline (pH 7.4). Filters were counted in a Microbeta Plus liquid scintillation counter (Wallac Inc.) at an average counting efficiency of 35%.

[3H]/DA Uptake Assay—The general conditions for conducting the uptake assays were as described by us previously for synaptosomal suspensions (22). Briefly, HEK-293-hDAT cells were lifted from the culture flasks with phosphate (10 mM)-buffered saline (pH 7.4) and centrifuged at 100 × g for 6 min. The pellet of cells from two confluent 150-cm$^2$ flasks was resuspended in 6 ml of saline in a glass homogenizer with a motor-driven Teflon pestle (clearance of approximately 0.15 mm compared with a cell diameter of 0.01 mm). This step helped to produce even suspensions while not causing more cell damage than resuspension through vortexing (combined with repeated movements through a 5-ml pipette) as judged under the microscope by the Trypan Blue dye exclusion test. Uptake assays were conducted in a final volume of 0.4 ml of the above described assay buffer at selected pH values and contained 50 μl of cell suspension, 20 μl of [3H]/DA stock (contributing a final 6 nM concentration of tritiated DA (NEN Life Science Products) and 44 nM unradioactive DA), and for saturation studies 20 μl of varying amounts of unlabeled WIN 35428 (100 nM to 10 μM) and for final concentrations ranging from 1 to 0.01 nM). Nonspecific uptake was defined by 100 μM cocaine. After a preincubation of the cell suspension and assay tubes with assay buffer for 15 min in a 21 °C water bath, 50 μl of cell suspension was added to the tubes, which were then incubated while gently shaking in a 21 °C water bath for 8 min. The reaction was terminated with the addition of 4 ml of ice-cold wash buffer (assay buffer minus troponol). Subsequent filtration (within 4 min after stopping for all tubes) was performed with a 24-pin Brandel harvester through a Whatman GF/C glass fiber filter presoaked in 0.05% (w/v) poly-l-lysine with three additional rinses of 4 ml of ice-cold wash buffer. In control experiments for assay mixtures with stop buffer, after 6 min on ice no efflux of accumulated [3H]DA was observed. The radioactivity on the filters was measured in 5 ml of CytoScint mixture with a Beckman model LS 6000IC scintillation counter with quench correction. The average protein content of the cell suspensions was estimated to be 250 μg determined by the method of Lowry described above.

**Distribution of Neutral and I onic Forms of DA at Varying pH**—The pH values were set to 6.0, 8.2, and the $pK_z$ values to 10.5, which is the average of the values of 8.8–8.9 and 10.4–10.6, respectively, reported by Armstrong and Barlow (10), Lewis (11), and Mack and Bonisch (12). The first $pK_z$ governs the transition from the cationic ‘H$_2$NDO to the mixture of ‘H$_2$NDOH+ and H$_2$NDOH (both overall neutral species) rather than representing the dissociation of the amino group only (10, 12). Similarly, the second $pK_z$ describes the transition of the two neutral species into the anion H$_2$NDO$^-$ and not just the dissociation of the hydroxyl group (10, 12). This analysis does not consider extremely basic pH conditions in which ionization could occur in the second hydroxyl group, which has a $pK_z > 12$.

The percent of total DA existing in the neutral form (‘H$_2$NDO$^+$ + H$_2$NDOH) as a function of pH was calculated according to Mack and Bo¨nisch (12) as follows: $% neutral = 100/1 + antilog(pK_a + antilog(pH − pK_a))$.

The percent of total DA existing in the cationic form was calculated as follows: $% positive = 100/(1 + antilog(pH − pK_a) + antilog(2pH − pK_a − pK_z))$; this was arrived at by rewriting the equations for the two equilibria described by Armstrong and Barlow (10) as shown for the single case of protonation of a base by Courtney and Strichartz (36). This derivation also takes into account that log((‘H$_2$NDO$^+$ + H$_2$NDOH/‘H$_2$NDO))/log(‘H$_2$NDOH/‘H$_2$NDO+ + H$_2$NDOH) = $pK_a$ − $pK_z$, by solving both equilibrium equations for pH.

The percent of total DA existing in the anionic form was calculated as follows: $% negative = 100 − % neutral − % positive$.

**Prediction of DA Uptake Velocity Based on the Neutral Form of DA Binding by Compound—**For binding by compounds, the $K_m$, $v_{max}$, and $V_{max}$ values for the neutral form were calculated (21) (“assay buffer”). The $K_m$ and $v_{max}$ values were obtained from the lines of the given set of pH values for each compound. For fixed $pK_z$ and $pK_a$ values, varying pH was predicted based on fixed $pK_a$, and $pK_z$ values for the case that the neutral form is the active form for translocation. The $pK_a$ was always 10.5 (see above), and the $pK_z$ was 8.86 (theoretical value, see above) or set at a value between 6.8 and 9.5 (see “Results” and “Discussion”). At pH 8.2, the highest pH studied, more DA will be in the neutral form than at a lower pH, and for each pair of $pK_a$ and $pK_z$, the $K_m$ and $V_{max}$ values for DA binding at each pH. All results are expressed as means ± S.E. Statistics included one-way ANOVA followed by the Student-Newman-Keuls multiple comparisons test or the least significant difference multiple range test and two-way ANOVA with factors A and B and interactions A × B. Where needed, data were log-transformed for homogeneity of variance. The accepted level of significance was 0.05.

**RESULTS**

**DA Uptake as a Function of pH**—The $K_m$ for [3H]DA uptake decreased approximately 3-fold upon increasing the pH from 6.0 to 7.4 and did not change upon further increasing the pH to 8.2 (Fig. 1A). There were no statistically significant differences in the $V_{max}$ values obtained in the 6.0–8.2 pH range.

The velocity of [3H]DA uptake at a fixed concentration of DA of 56 nM increased sharply upon increasing the pH from 6.0 to 7.4.
7.0 and then remained at a plateau up to the highest pH applied, which was 8.2 (Fig. 1B).

A different pH dependence was calculated for the concentration of either the cationic form of DA (\(\text{H}_3\text{NDOH}\)), which at a \(pK_{a1}\) of 8.86 and \(pK_{a2}\) of 10.5 (10–12), was in the majority at all pH values studied, or the neutral forms (zwitterion \(\text{H}_3\text{NDO}^2+\) plus neutral form \(\text{H}_2\text{NDOH}\)), which increased approximately 10-fold for each pH unit increase (Fig. 1B). Indeed, velocity predictions based on the neutral form being the substrate deviated substantially from the observed pH dependence of DA uptake (Fig. 1B, compare solid line with data points with broken line for \(pK_{a1} = 8.86\)).

**DA Binding as a Function of pH**—The \(K_v\) value for DA in inhibiting \(^3\text{H}\)WIN 35428 binding decreased approximately 3-fold upon increasing the pH from 6.0 to 7.4 and showed a smaller decrease of approximately 1.5-fold upon further increasing the pH to 8.2 (Fig. 1A).

**Guanethidine and Bretylium Binding as a Function of pH**—The affinity of guanethidine for the DAT was generally lower than that of DA (for \(K_v\) values see legend to Fig. 2). However, there was a resemblance between guanethidine and DA in the pattern of potency changes as a function of pH (Fig. 2). Thus, the \(K_v\) value for guanethidine in inhibiting \(^3\text{H}\)WIN 35428 binding decreased approximately 3-fold upon increasing the pH from 6.0 to 7.4 and showed a smaller decrease of approximately 2-fold upon further increasing the pH to 8.2 (Fig. 2). WIN 35428 itself had a higher potency than DA or guanethidine (see legend to Fig. 2), but again the pattern of pH dependence was similar (Fig. 2) (note the statistically significant interaction factor in analysis of variance of all compounds including bretylium).

No evidence was found for a deviation from a competitive mechanism of inhibition of \(^3\text{H}\)WIN 35428 binding by bretylium or guanethidine. First, the Hill numbers for the inhibition
DA uptake data could be interpreted in such a context with a value of 8.4 – 8.7 measured in water (41). The present pattern at the pH 6.0, 0.001).

$\text{K}_D$ values were determined from inhibition curves for each compound as described under “Experimental Procedures.” Each point represents the mean ± S.E. (vertical bar, shown where greater than point itself) of three independent experiments carried out in triplicate expressed as the percent of the value at pH 7.4. At this pH level, the $\text{K}_D$ value for WIN 35425 was 15 nm; for DA, 6.0 μm; for guanethidine, 327 μm; and for bretylium, 664 μm. * indicates $p > 0.05$ for interaction factor (F(9, 28) = 2.68) (two-way ANOVA with pH as factor A and inhibitors guanethidine, DA, and WIN 35425 as factor B; both factors were highly significant with $p < 0.001$). $\dagger$ indicates $p < 0.001$ for factor B (F(2, 9) = 6.72) (two-way ANOVA with pH as factor A and inhibitors bretylium, guanethidine, DA, and WIN 35425 as factor B; both factors were highly significant with $p < 0.001$).

Predicted DA Uptake with Overall Neutral DA as the Substrate—If it is assumed that the increase in DA uptake with pH is accounted for solely by the shift in the distribution of DA forms toward the overall neutral forms (H$_2$NDOH$^-$), the predicted pH dependence does not coincide with the observed pattern at the pH values reported for DA of 8.86 and 10.5, respectively (10 – 12). It could be argued that the $pK_{a,1}$ value may be reduced in the vicinity of the binding site when its environment is more hydrophobic, making it easier for the compound to loose a proton. This pattern has been suggested for the binding of cocaine to the local anesthetic binding site in the voltage-dependent sodium channel, which is predicted more accurately by a $pK_a$ value of 7.1 than the value of 8.4 – 8.7 measured in water (41). The present DA uptake data could be interpreted in such a context with a $pK_{a,1}$ value of 6.8 resulting in a reasonable fit for uptake at a fixed concentration of 56 nm [H]$^+$DA in the studied pH range of 6.0 – 8.2. Another way the possibility of a $pK_a$ shift can be shown is by calculating the $K_m$ or $K_I$ of the neutral forms of DA from the observed “composite” $K_{m,1}$ taking into account the proportion of the neutral forms at each pH. If the calculated values remain constant across pH, this result would be in agreement with the concept that pH-dependent changes in proportion solely underlie the uptake or binding changes. At pH 6.0, the $K_{m,1}$ of the neutral forms was not constant across pH, but lowering the $pK_{a,1}$ to 6.8 gave reasonably constant values (Table I).

It can only be hypothesized as to how hydrophobic the environment is around the DA binding site to substantially reduce the $pK_{a,1}$ value below that found in a water milieu. DA is thought to bind to a site in a central aqueous cavity surrounded by amphipathic membrane-spanning helices (5). Transporter chimera and truncation studies support the idea that both substrates and blockers interact with residues in transmembrane domains lining the permeation pathway (reviewed in Ref. 4), which may include Asp-79 in transmembrane domain 1 (15). Prior to translocation, DA accesses its centrally located binding site, which is facing externally; translocation occurs through a conformational change exposing the binding site on the internal face (5, 42). The presently observed similarity in the pH dependence of DA uptake and binding suggests that the pH effects occur at the level of DA recognition. It is possible that the DA binding site in the central crevice of the DAT is accessed by DA from the aqueous side, whereas the local anesthetic site near the inactivation gate in the Na$^+$ channel (43) can be reached by the relatively more lipophilic cocaine (log P of 2.4 (44)) compared with –0.99 for DA (12)) through the hydrophobic pathway in the membrane lipidic environment (45). Recent evidence for the serotonin transporter, which is closely related to the DAT, suggests binding of serotonin to Ile-72 and Tyr-176 (46). These residues lie in the $\alpha$-helical structure of transmembrane domain 3 on a vertical patch that faces toward the binding pocket accessible to the external medium as judged from their sensitivity to membrane-impermeant, charged [2-(trimethylammonium)ethyl]methanethiosulfonate in the cysteine-scanning approach (46).

Predicted DA Uptake with Neutral, Uncharged DA as the Substrate—The composition of the mixture of the zwitterion H$_3$NDO$^+$ and the neutral form H$_2$NDOH is defined by the

| pH | $K_p$ (nM)* | $K_D$ (pmol/mg protein)* | $B_{max}$ (pmol/mg protein)* |
|----|-------------|--------------------------|-------------------------------|
| 6.0 | 158 ± 13    | 255 ± 32                 | 315 ± 43                    |
| 8.2 | 5.13 ± 0.36 | 5.29 ± 0.73              | 5.41 ± 0.58                 |

| pH | $K_p$ (nM)* | $K_D$ (pmol/mg protein)* | $B_{max}$ (pmol/mg protein)* |
|----|-------------|--------------------------|-------------------------------|
| 6.0 | 5.74 ± 0.66 | 5.43 ± 1.10              | 5.63 ± 1.26                 |

* $p < 0.01$ (F(2, 18) = 12.65 for factor B in two-way ANOVA with pH as factor A and added drug as factor B).

** $p > 0.01$ (compared with control at same pH; least significant difference multiple range test following one-way ANOVA at same pH indicating significant differences (F(2, 9) = 7.95; $p < 0.01$)).

# $p > 0.9$ (F(2, 18) = 0.018 for factor B in two-way ANOVA with pH as factor A and added drug as factor B).

$^\dagger$ $p < 0.05$ (compared with guanethidine at same pH; least significant difference multiple range test following one-way ANOVA at same pH indicating significant differences (F(2, 9) = 10.36; $p = 0.005$)).
equilibrium dissociation constant for DA uptake is approximately 7.83 (the average of the range 5.8–10.0) by various approaches (10). Thus, the neutral, uncharged form is less prevalent than the zwitterion, but its increase as a function of rising pH is the same as for the total mix of \( \text{H}_2\text{NDO}^+ \)/\( \text{H}_2\text{NDOH}^- \). Indeed, when the DA uptake velocity at 56 nM \([3H]DA\) was calculated using the mean \( K_m \) and the amount of DA in the water environment.

**Predicted DA Uptake with Zwitterionic DA as the Substrate.—** The increase of the zwitterion as a function of rising pH is the same as for the neutral form of DA and again does not fit the experimental data at pH 7.4. Therefore, the percentage change in the uptake velocity from observed inhibition by DA of \([3H]\text{WIN 35428} \) binding at pH 7.4 is typically high compared with previous results obtained by rapid filtration, and yet \([3H]DA\) binding to the DAT has never been observed. Clearly, the hypothesis that anionic DA is the substrate for the DAT is untenable.

**Predicted DA Uptake with Cationic DA as the Substrate.—** Most of the DA (at \( K_{p_{an}} \) 8.86 and \( K_{p_{an}} \) 10.5) was in the cationic form over the pH range studied, slightly decreasing from 99.9% at pH 6.0 to 82.0% at pH 8.2. For example, the IC \(_{50}\) value of bretylium at pH 7.4 of 664 \( \mu M \) is appreciably higher than the IC \(_{50}\) value of 149 \( \mu M \) reported by Kammerer et al. (29) for inhibition of striatal synaptosomal DA uptake. The likely reason for this difference is that bretylium, like DA, is a substrate for the DAT, requiring higher concentrations for saturation of binding than uptake. The same

### Table II

**Estimates of the \( K_m \) or \( K_I \) of the neutral form of DA as substrate computed from the observed “composite” \( K_m \) or \( K_I \) at varying pH (see text)**

| pH  | \( \text{pK}_{an} \) | \( K_m(\text{neutral}) \) | \( K_m(\text{composite}) \) | \( K_I(\text{neutral}) \) | \( K_I(\text{composite}) \) |
|-----|----------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| 6.0 | 8.86 | 1.4 | 3.75 | 8.52 | 6.4 |
| 6.5 | 8.77 | 7.2 | 3.56 | 6.5 | 6.4 |
| 7.4 | 8.2 | 64 | 36.5 | 52.6 | 6 |
| 8.2 | 5.7 | 22 | 30 | 6 | 6 |

The observed \( K_f \) of bretylium at pH 7.4 of 664 \( \mu M \) is appreciably higher than the IC \(_{50}\) value of 149 \( \mu M \) reported by Kammerer et al. (29) for inhibition of striatal synaptosomal DA uptake. The likely reason for this difference is that bretylium, like DA, is a substrate for the DAT, requiring higher concentrations for saturation of binding than uptake. The same
may apply to guanethidine, which has a high $K_v$ value of 327 $\mu$m at pH 7.4.

Comparison with Other Transporters for Monoamines—The present data are consonant with the conclusion that the cationic form of DA, perhaps including the zwitterion, is the more likely substrate for the DAT. Accordingly, the results are consistent with the assumption that translocation of one DA molecule brings in one positive charge (9). pH dependence studies have led to a similar conclusion for the transport of serotonin by the plasma membrane serotonin transporter (13) and of norepinephrine by the plasma membrane norepinephrine transporter (14). For transport of monoamines by the vesicular monoamine transporter, the issue is still being debated (5). Although the majority of the earlier studies favored the idea of the neutral forms of monoamines acting as substrates (for the vesicular monoamine transporter, the issue is still being debated (5). For transport of monoamines by the DAT, which may readily carry protons or otherwise be sensitive to pH changes, possibly by a pH-dependent ion selectivity (9).

REFERENCES

1. Zimanyi, I., Lajtha, A., and Reith, M. E. A. (1989) Naunyn-Schmiedeberg's Arch. Pharmacol. 340, 626–632
2. Garris, P. A., Ciolkowski, E. L., Pastore, P., and Wightman, R. M. (1994) J. Neurosci. 14, 6084–6093
3. McElvain, J. S., and Schenk, J. O. (1992) Biochem. Pharmacol. 43, 2189–2199
4. Pavlicek, S., and Amara, S. S. (1997) in Neurotransmitter Transporters: Structure, Function, and Regulation (Reith, M. E. A., ed) pp. 1–8, Humana Press, Totowa, NJ
5. Rudnick, G. (1997) in Neurotransmitter Transporters: Structure, Function, and Regulation (Reith, M. E. A., ed) pp. 73–100, Humana Press, Totowa, NJ
6. Gu, H. H., and Rudnick, G. (1996) Soc. Neurosci. Abstr. 22, 370
7. Krueger, B. K. (1990) J. Neurochem. 55, 260–267
8. Gu, H., Wall, S. C., and Rudnick, G. (1994) J. Biol. Chem. 269, 7124–7130
9. Sonders, M. S., Zhu, J. S., Zahniser, N. R., Kavanaugh, M. P., and Amara, S. G. (1997) J. Neurosci. 17, 960–974
10. Armstrong, J., and Barlow, R. B. (1976) Br. J. Pharmacol. 57, 501–516
11. Lewis, G. P. (1954) Br. J. Pharmacol. Chemother. 9, 488–493
12. Mack, F., and Bonisch, H. (1979) Naunyn-Schmiedeberg's Arch. Pharmacol. 310, 1–9
13. Rudnick, G., Kirk, K. L., Fishkes, H., and Schuldiner, S. (1989) J. Biol. Chem. 264, 14865–14868
14. Gu, H. H., Wall, S., and Rudnick, G. (1996) J. Biol. Chem. 271, 6911–6916
15. Kizawa, S., Shimada, S., Xu, H., Markham, L., Denovan, M. D., and Uhl, G. R. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 7783–7787
16. Edvardsen, O., and Dahl, S. G. (1994) Mol. Brain Res. 237, 265–274
17. Njus, D., Kelley, P. M., and Harmadek, G. J. (1986) Biochem. Biophys. Acta 853, 267–265
18. Darchen, F., Scherman, D., Desnos, C., and Henry, J. P. (1988) Biochem. Pharmacol. 37, 4381–4387
19. Johnson, R. G. J. (1988) Physiol. Rev. 68, 322–307
20. Eshleman, A. J., Henningsen, R. A., Neve, K. A., and Janowsky, A. (1994) Mol. Pharmacol. 45, 312–316
21. Eshleman, A. J., Stewart, F., Erenson, A. K., Mason, J. N., Blakely, R. D., Janowsky, A., and Neve, K. A. (1997) J. Neurochem. 69, 1459–1466
22. Xu, C., Coffey, L. L., and Reith, M. E. A. (1995) Biochem. Pharmacol. 49, 339–350
23. Reith, M. E., Xu, C., Zhang, L., and Coffey, L. L. (1996) Naunyn-Schmiedeberg's Arch. Pharmacol. 354, 295–304
24. Reith, M. E. A., de Costa, B., Rice, K. C., and Jacobson, A. E. (1992) Eur. J. Pharmacol. 237, 417–424
25. Maxwell, R. A., Ferris, R. M., and Bureus, J. E. (1976) in The Mechanism of Neuronal and Extraneuronal Transport of Catecholamines (Paton, D. M., ed) pp. 95–153, Raven Press, New York
26. Manuela, M., Kanai, S., and F. (1998) Am. J. Physiol. 274, C29–C34
27. Molinoff, P. B., Brumisom, J., and Axelrod, J. (1971) Biochem. Pharmacol. 169, 132–133
28. Kamel, A. A., Arbilla, S., Galzin, A. M., and Langer, S. Z. (1983) Biochem. Pharmacol. 32, 237–265
29. Kammerer, R. C., Amiri, B., and Cho, A. K. (1979) J. Med. Chem. 25, 352–355
30. Steinmetz, P. R., and Balc, C. (1973) J. Neurobiol. 146–146
31. Katzung, B. G. (1995) in Basic & Clinical Pharmacology (Katzung, B. G., ed) pp. 1–8, Appleton & Lange, Norwalk, CT
32. Collins, G. G., and West, G. B. (1988) Br. J. Pharmacol. 54, 514–522
33. Silinsky, E. M. (1991) Br. J. Pharmacol. 51, 367–371
34. Sartore, C. (1970) Acta Physiol. Scand. 34, 1–66
35. Wu, Q., Coffey, L. L., and Reith, M. E. A. (1997) J. Neurochem. 69, 1106–1118
36. Courtney, R. C., and Strichartz, G. R. (1987) in Handbook of Experimental Pharmacology (Strichartz, G. R., ed) Vol. 31, pp. 83–94, Springer-Verlag.
Berlin
37. DeLean, A., Munson, P. J., and Rodbard, D. (1978) *Am. J. Physiol.* 235, E97–E102
38. Munson, P. J., and Rodbard, D. (1980) *Anal. Biochem.* 107, 220–239
39. Xu, C., and Reith, M. E. (1996) *J. Pharmacol. Exp. Ther.* 278, 1340–1348
40. Cheng, Y., and Prusoff, W. H. (1973) *Biochem. Pharmacol.* 22, 3099–3108
41. Nettleton, J., and Wang, G. K. (1990) *J. Physiol.* 529, 95–106
42. Amejdki-Chab, N., Benmansour, S., Costentin, J., and Bonnet, J. J. (1992) *J. Neurochem.* 59, 1795–1804
43. Taylor, C. P., and Meldrum, B. S. (1995) *Trends Pharmacol. Sci.* 16, 309–316
44. Leo, A., Hansch, C., and Elkins, D. (1971) *Chem. Rev.* 71, 525–616
45. Hille, B. (1984) *Ionic Channels of Excitable Membranes*, pp. 287–289, Sinauer Associates, Inc., Sunderland, MA
46. Chen, J.-G., Sachpatzidis, A., and Rudnick, G. (1997) *J. Biol. Chem.* 272, 2821–2827
47. Meiergerd, S. M., and Schenk, J. O. (1994) *J. Neurochem.* 62, 988–1008
48. Wall, S. C., Innis, R. B., and Rudnick, G. (1993) *Mol. Pharmacol.* 43, 264–270
49. Bonnet, J. J., Benmansour, S., Vaugeois, J. M., and Costentin, J. (1988) *J. Neurochem.* 50, 759–765
50. Amejdki-Chab, N., Costentin, J., and Bonnet, J. J. (1992) *J. Neurochem.* 58, 799–805
51. Andersen, P. H. (1987) *J. Neurochem.* 48, 1887–1896