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Presence of Base-Exchange Activity in Rat Brain Nerve Endings: Dependence on Soluble Substrate Concentrations and Effect of Cations

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Abstract: The calcium-dependent, energy-independent incorporations of ^{14}C-labeled bases, choline, ethanolamine, and serine, into their corresponding membrane phospholipids, phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine, were compared in microsomes and in subcellular fractions prepared from a lysed crude mitochondrial (P2) pellet of whole rat brain. When activities were measured in the presence of an extracellular (1.25 m*M*) concentration of Ca^{2+}, recovered activities were highest in the microsomal fraction, although substantial activity remained associated with the P2 homogenate even after repeated washing of the pellet. When this washed P2 homogenate was subfractionated, enrichment of all three exchange activities was obtained only in a fraction that was fivefold enriched over the homogenate and sevenfold enriched over the microsomal fraction in Na^+, K^+-ATPase, a plasma membrane marker. This strongly suggests that the base-exchange enzymes are normal constituents of synaptosomal plasma membranes. The three exchange activities were measured in synaptosomes prepared from whole rat brain in the presence of various substrate (base) concentrations, and kinetic constants were calculated. The V_{max} values for choline, ethanolamine, and serine exchange were, respectively, 1.27 ± 0.09, 1.60 ± 0.17, and 0.56 ± 0.06 nmol/mg of protein/h; the respective K_m (apparent) values were 241 ± 29, 65 ± 18, and 77 ± 22 μM. Endogenous levels of the three bases, choline, ethanolamine, and serine, in whole (microwaved) rat brains were 20 ± 8, 78 ± 28, and 639 ± 106 nmol/g, respectively. That ethanolamine and serine incorporations had lower K_m values than choline incorporation suggests that these bases are preferentially incorporated into their respective phospholipids. Moreover, that endogenous choline levels are well below those needed for enzyme saturation suggests that the incorporation of choline into phosphatidylcholine via this pathway may be sensitive to transitory increases in free choline concentration (such as occur intrasynaptically after acetylcholine is released and hydrolyzed). Various polyvalent cations (Mg^{2+}, Ba^{2+}, Ni^{2+}, Co^{2+}, and La^{3+}) inhibited synaptosomal base-exchange activity with a rank order of potency similar to their ability to block calcium channels. **Key Words:** Base-exchange—Choline—Ethanolamine—Serine—Phosphatidylcholine—Calcium. **Holbrook P. G. and Wurtman R. J.** Presence of base-exchange activity in rat brain nerve endings: Dependence on soluble substrate concentrations and effect of cations. *J. Neurochem.* **50,** 156–162 (1988).

Membrane fractions isolated from mammalian and avian brain possess catalytic activities attributed to an enzyme or set of enzymes called the base-exchange enzymes (Porcellati et al., 1971; Kanfer, 1972). This activity enables membranes to incorporate radiolabeled nitrogenous "bases," choline, ethanolamine, and serine, into preformed phospholipids of unspecified chemical composition, yielding the corresponding phospholipids, phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylserine (PS). The base-exchange pathway is distinct from the de novo pathway for phospholipid biosynthesis, described by Kennedy and Weiss (1956), which incorporates choline or ethanolamine into PC or PE, respectively, via a cytidyl diphosphoryl intermediate: The former requires calcium, does not re-

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Abbreviations used: HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; SPM, synaptosomal plasma membrane.

quire metabolic energy, and is able to incorporate serine, as well as choline or ethanolamine, into membrane phospholipids.

When measured in the presence of high "extracellular" calcium concentrations (2–25 m*M*), base-exchange activity is enriched in microsomal brain fractions (Porcellati et al., 1971; Buchanan and Kanfer, 1980*a;* Hattori and Kanfer, 1985) and has been most extensively characterized in this fraction (Porcellati et al., 1971; Kanfer, 1972; Butler and Morell, 1983; Corazzi et al., 1986). Studies using rabbit brain concluded that base-exchange could be used as a neuronal marker, on the basis of data (Goracci et al., 1973) indicating that neurons possess higher serine and ethanolamine exchange activity than glia and that ethanolamine exchange activity associated with the neuronal plasma membrane is slightly greater than that observed in neuronal microsomes. Some evidence suggests that synaptosomes also are able to incorporate choline into PC via this pathway (Miller and Dawson, 1972) and that this process may be of particular importance within cholinergic nerve endings (Abdel-Latif and Smith, 1972).

The following studies compare the base-exchange activity in synaptosomal plasma membrane (SPM) with that in other subcellular fractions of whole rat brain and, in addition, characterize the kinetic aspects and effects of cations on the three exchange reactions in synaptosomes.

EXPERIMENTAL PROCEDURES

Animals

Male Sprague-Dawley rats (weighing 150–220 g; Charles River) were housed under light (Vita-Lite, Duro Test) for 12 h daily and given free access to food (Charles River rat chow) and water. Animals were killed by decapitation, and their brains were quickly removed and maintained in 0.32 *M* sucrose on ice until homogenized and assayed. In some experiments, animals were killed by fixed microwave irradiation to the head to denature proteins rapidly and thereby arrest the postmortem increases in amounts of free "bases" that might otherwise have occurred because of enzymatic hydrolysis of membrane phospholipids.

Materials

Special chemicals used in these experiments were obtained from the following sources: radioactive bases from ICN (Irvine, CA, U.S.A.) or Amersham (Arlington Heights, IL, U.S.A.); TLC plates (LK5-D) from Whatman Co. (Clifton, NJ, U.S.A.); phospholipid standards from SUPELCO (Bellefonte, PA, U.S.A.); and scintillation cocktail from National Diagnostics (Somerville, NJ, U.S.A.). All other chemicals used were reagent grade or better.

Preparation of tissue fractions

SPMs, mitochondria, and myelin were prepared from whole rat brain (2.0–2.5 g) by the method of Jones and Matus (1974). A well-washed, crude mitochondrial (P2) pellet was hypotonically lysed in a buffer consisting of 5 m*M* *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), 1 m*M* NaH_2PO_4, and 0.1 m*M* EDTA (pH 8.0), and the major membrane fractions (SPM, mitochondria, and myelin) were separated by simultaneous sedimentation and flotation centrifugation on discontinuous sucrose gradients made up in the same buffer. The composition of the buffer was chosen for the following reasons: low ionic strength is required to achieve lysis of synaptosomes; a slightly alkaline pH is necessary to achieve good separation of mitochondria from SPM (Jones and Matus, 1974); buffers containing 1 m*M* phosphate and 0.1 m*M* EDTA preserve Na^+, K^+-ATPase activity (Gurd et al., 1974); and the presence of EDTA in the buffer prevents loss of base-exchange activity (Buchanan and Kanfer, 1980*b*). Microsomes were prepared as described by Gurd et al. (1974).

Synaptosomes were prepared from whole rat brain by the method of Dodd et al. (1981). Whole brains (2.0–2.5 g) were homogenized in 10 volumes of 0.32 *M* sucrose and centrifuged at 1,000 *g* at 0°C for 15 min so that cell debris and nuclei were removed. The supernatant was then layered onto 4 ml of 1.2 *M* sucrose and centrifuged at 130,000 *g* at 0°C for 15 min. The pellet contained mitochondria; synaptosomes, myelin, and some microsomes were retained at the interface. The interface, containing synaptosomes and myelin, was diluted in 0.32 *M* sucrose, layered onto 4 ml of 0.8 *M* sucrose, and centrifuged as above for 15 min. The resulting pellet contained synaptosomes, whereas myelin was retained at the interface and microsomes remained suspended mainly in the upper phase. The synaptosomal preparation, characterized by electron microscopy, consisted largely of intact vesicles 0.7–1.3 mm in diameter. The preparation contained few empty vesicles or free mitochondria.

Base-exchange assay

Base-exchange activity was assayed by a modification of the method described by Kanfer (1972) using an assay medium (final volume of 200 μl) comprising 50 m*M* HEPES buffer (pH 7.6), 0.32 *M* sucrose, 1.25–2.5 m*M* $CaCl_2$, 50–200 μg of synaptosomal protein, and 0.10–0.50 μCi of radioactive base. The reaction was carried out at 37°C for 15–20 min in a Dubnoff shaker and stopped by addition of 3 ml of the extraction solvent (chloroform/methanol 2:1 vol/vol). A zero-time blank was used as the reaction blank in all experiments. Samples were extracted by the method of Folch et al. (1951) at 4°C overnight and washed twice with 2 ml of 0.75% *M* KCl in 50% methanol the following day. The aqueous phases were removed by aspiration, and the final organic phase was dried in a Savant-Speed-Vac lyophilizing centrifuge. Sample extracts were reconstituted in 100 μl of chloroform/methanol (1:1 vol/vol), and the reaction products were identified as described below.

Identification of reaction products

Phospholipid reaction products were identified by TLC. Sample extracts and phospholipid standards were applied to silica gel TLC plates (Whatman LK5-D) and developed using either chloroform/methanol/acetic acid/water (25:15:4:2 vol/vol) (Kanfer, 1972) or chloroform/methanol/isopropanol/0.25% KCl/triethylamine (30:9:25:6:18 vol/vol) (Touchstone et al., 1979) as the mobile phase system. Phospholipids were visualized by UV light [after exposure to diphenylhexatriene spray (0.1% diphenylhexatriene in petroleum ether) (Hyslop and York, 1980)]; PE and PS were preferentially visualized with ninhydrin spray (0.3% ninhydrin and 3% acetic acid in *n*-butanol). The appropriate bands were scraped into scintillation vials: The ^{14}C-labeled phospholipids were then eluted with 1 ml of methanol, and their radiation was counted using a Beck-

TABLE 1. *Base-exchange activity in SPMs and other subcellular fractions of whole rat brain*

Fraction	Protein	Markers			Base-exchange enzymes					
					Choline		Ethanolamine		Serine	
		RNA	NADPH-cytochrome c reductase	Na^+,K^+-ATPase	100 μM	500 μM	100 μM	500 μM	100 μM	500 μM
P2 Homogenate										
1st wash	156 ± 36	5.1 ± 0.3	1.9 ± 0.2	19 ± 4	—	1.2 ± 0.2	—	3.3 ± 0.9	—	1.0 ± 0.2
2nd wash	113 ± 33	—	2.0 ± 0.7	17 ± 2	—	0.7 ± 0.2	—	2.3 ± 0.8	—	0.8 ± 0.3
3rd wash	82 ± 18	—	3.3 ± 1.0	18 ± 4	0.3 ± 0.1	0.6 ± 0.1	1.9 ± 0.4	1.7 ± 0.6	0.5 ± 0.20	0.7 ± 0.2
Myelin	12 ± 3	—	ND	6 ± 1	0.1 ± 0.04	0.1 ± 0.03	0.6 ± 0.04	0.6 ± 0.1	0.2 ± 0.03	0.2 ± 0.1
SPM	4 ± 0	ND	3.4 ± 1.2	96 ± 6	0.6 ± 0.03	1.0 ± 0.4	3.4 ± 0.3	2.7 ± 0.3	1.1 ± 0.1	1.3 ± 0.4
Mitochondria	18 ± 2	—	1.7 ± 0.1	9 ± 3	0.2 ± 0.1	0.3 ± 0.1	1.4 ± 0.1	1.5 ± 0.5	0.3 ± 0.01	0.5 ± 0.1
Microsomes	48 ± 13	16.7 ± 1.8	10.0 ± 1.1	14 ± 1	2.3 ± 0.2	5.5 ± 1.0	7.2 ± 0.5	10.3 ± 1.2	2.6 ± 0.1	4.2 ± 1.0

Data are mean ± SD values from three to four determinations made in two or three separate experiments. Protein content is expressed as mg/fraction and represents the yield from two brains. RNA content is expressed as μg of RNA/mg of protein. NADPH-cytochrome c reductase activity is expressed as nmol/mg of protein/min. Na^+,K^+-ATPase activity is expressed as μmol of phosphorus/mg of protein/h. Base-exchange enzyme activity is expressed as nmol/mg of protein/h in the presence of the indicated concentration of base. ND, not detected.

man LS 7500 scintillation spectrophotometer, after addition of 10 ml of scintillation fluor. The cpm values were converted to dpm values with correction for quenching using the H number.

Other assays

Protein content was determined by the method of Lowry et al. (1951). RNA content was determined by the method of Fleck and Begg (1965). Ouabain-sensitive Na^+, K^+-ATPase activity was determined by the method of Post and Sen (1967). NADPH-cytochrome c reductase activity was assayed as described by Sottocasa et al. (1967). Choline content was measured by a radioenzymatic assay (Goldberg and McCaman, 1973). Serine and ethanolamine contents were measured by reversed-phase HPLC with fluorometric detection (Fernstrom and Fernstrom, 1981).

Data analysis

For studies of enzyme kinetics, theoretical binding curves were fitted to the experimental data points by nonlinear, least-squares regression using a computer program, the RS1 Software Package.

RESULTS

Comparison of base-exchange activity in subcellular fractions of whole rat brain

Base-exchange activity in the microsomal fraction was compared with that in subfractions of a lysed crude mitochondrial (P2) pellet prepared from whole rat brain at both saturating (500 μM) and subsaturating (100 μM) base concentrations (Table 1). The subfractions were characterized by measuring RNA content and NADPH-cytochrome c reductase activity as microsomal markers and ouabain-sensitive Na^+, K^+-ATPase activity as a plasma membrane marker. RNA content and NADPH-cytochrome c reductase activity in the microsomal fraction were three- to fourfold enriched over values in the P2 homogenates. Na^+, K^+-ATPase activity in SPM was fivefold enriched over that in the P2 homogenate and was sevenfold greater than the activity observed in microsomes. As has been reported previously (Gurd et al., 1974), the SPM fraction possessed detectable NADPH-cytochrome c reductase activity, which was one-third of that observed in the microsomal fraction, even though the content of the microsomal marker RNA, a marker for rough endoplasmic reticulum and ribosomes, was very low or not detectable. NADPH-cytochrome c reductase in SPM was not enriched over that in the starting P2 pellet.

When measured in the presence of extracellular (1.25 mM) Ca^{2+} concentrations, base-exchange activity was highest in the microsomal fraction (Table 1). There was detectable base-exchange activity associated with the P2 homogenate even after repeated washing of the pellet (Table 1), and this activity appears to be a component of the SPM, because it was enriched over the value in the starting homogenate only in this fraction (Table 1).

Base-exchange kinetics in synaptosomes

Synaptosomes incorporated ^{14}C-labeled choline, ethanolamine, or serine into PC, PE, and PS, respectively, in a manner consistent with mediation by base-exchange reactions (Kanfer, 1972). These incorporations required calcium, were energy independent, and occurred optimally at an alkaline pH (data not shown). Enzyme activities in synaptosomes were examined as a function of substrate (base) concentrations, in three separate experiments (Table 2), and theoretical binding curves were fitted to the data points as described above. (An example, with data points and fitted line, appears in Fig. 1.) Choline has a lower affinity for its enzyme than either ethanolamine or serine (Table 2).

Levels of free choline, ethanolamine, and serine in brain samples taken from animals killed by focussed microwave beam were compared with those from rats killed by decapitation (Table 3). Free choline and

TABLE 2. *V_{max} and K_m (apparent) for base-exchange reactions in synaptosomes prepared from rat brain*

Substrate	$V_{max} \times 10^{-9}$ (mol/mg protein/h)	$K_m \times 10^{-6}$ (M)
Choline	1.27 ± 0.09	241 ± 29
Ethanolamine	1.60 ± 0.17	65 ± 18
Serine	0.56 ± 0.06	77 ± 22

The assay system was identical to that described in Experimental Procedures, except that unlabeled base was added to the reaction mixture to achieve the following substrate concentrations: 0.025, 0.05, 0.100, 0.250, 0.500, 1.0, and 2.0 mM for choline exchange and 0.02, 0.05, 0.10, 0.50, 1.0, and 1.5 mM for ethanolamine and serine exchange. Each substrate concentration was assayed in duplicate on three occasions. Radiolabeled bases of the following specific activities were added to the incubation medium: [*methyl*-^{14}C]choline chloride at 50 mCi/mmol, [1,2-^{14}C]ethanolamine hydrochloride at 95 mCi/mmol, and L-[U-^{14}C]serine at 165 mCi/mmol. For choline base-exchange, 0.25 μCi of label was added at substrate concentrations of ≤0.10 mM and 0.50 μCi at substrate concentrations of >0.10 mM. Similarly, for ethanolamine and serine exchange, 0.10 μCi was added at substrate concentrations of ≤0.10 mM and 0.25 μCi at substrate concentrations of >0.10 mM. Serine incorporation was saturated at 0.50 mM and demonstrated substrate inhibition at higher concentrations; therefore, these values were excluded from the analysis. Data were analyzed by computerized fit (nonlinear regression) to the function $V = V_{max}[S]/(K_m + [S])$. Correlation coefficients were ≥0.967. Data are mean ± SEM values from three experiments.

ethanolamine levels were lower in brains from microwaved than from nonmicrowaved animals, an observation indicating that both bases are subject to rapid postmortem increases in content, possibly reflecting hydrolysis of membrane phospholipids. Free serine levels in brains of microwaved rats were much higher than those of free choline or ethanolamine and

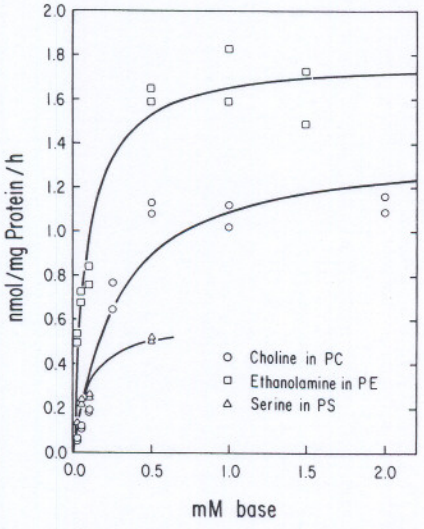

FIG. 1. Substrate kinetics of calcium-dependent base-exchange reactions in synaptosomes prepared from whole rat brain. Assay conditions were identical to those described in Table 1 and represent data from one experiment. The solid lines represent the theoretical fit to the data points indicated.

TABLE 3. *Levels of nitrogenous bases in whole rat brain*

	Choline	Ethanolamine	Serine
Microwaved	20 ± 8[a]	78 ± 28[b]	639 ± 106
Nonmicrowaved	49 ± 7	157 ± 60	654 ± 109

Choline, ethanolamine, and serine levels were measured as described in Experimental Procedures. Data are mean ± SEM values (in nmol/g) from five rats.

The microwaved groups were compared with their corresponding nonmicrowaved groups by a paired t test: [a] $p < 0.01$, [b] $p < 0.05$.

were not significantly affected by the mode of death (Table 3). This suggests that brain serine levels reflect general amino acid metabolism, rather than phospholipid turnover per se.

We also measured levels of free choline, ethanolamine, and serine in an incubation medium containing 200 μg of synaptosomal protein. At zero-time, these values were 3, 9, and 16 μM, respectively (data not shown) and varied both with time and calcium concentrations. There was a striking increase in content of serine and tyrosine (amino acids that would be derived from the breakdown of proteins) as the calcium concentration was raised to 20 mM, a result suggesting that calcium-dependent proteases had been activated. For this reason, we maintained calcium concentrations within the physiological range in all subsequent experiments.

Effects of ions

The effects of various cations on synaptosomal base-exchange reactions were tested using concentrations commonly used to block neurotransmitter release and voltage-dependent calcium channels (Hagiwara and Byerly, 1981) (Fig. 2). At the calcium concentrations used in these experiments (2.5 mM), alkaline earth cations (Mg^{2+} and Ba^{2+}) (5 mM) inhibited incorporation of labeled choline, but not of ethanolamine or serine, into phospholipids. At lower calcium concentrations, Mg^{2+} and Ba^{2+} also inhibited the incorporation of ethanolamine and serine (data not shown). The di- and trivalent metal cations Ni^{2+}, Co^{2+}, and La^{3+} were potent inhibitors of all three activities, with La^{3+} being the most potent.

DISCUSSION

These studies demonstrate that all three "bases" (choline, ethanolamine, and serine) are incorporated into synaptosomal and SPM phospholipids, by reactions that have characteristics of the base-exchange pathway. Previous studies have shown that synaptosomes prepared from brain of the rat (Abdel-Latif and Smith, 1972) and guinea pig (Miller and Dawson, 1972) incorporate choline into PC by base-exchange. It has been suggested that base-exchange activity in rat brain synaptosomes could reflect microsomal contamination (Buchanan and Kanfer,

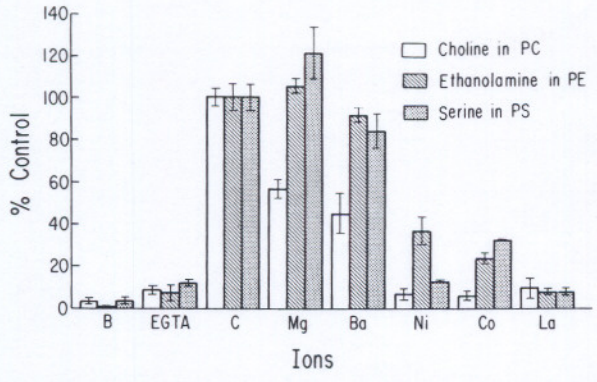

FIG. 2. Effect of polyvalent cations on synaptosomal base-exchange activity. Assay conditions were identical to those described in Experimental Procedures except that ions (5 m*M*, chloride salt) were added as indicated. Data are mean and ±SD (bars) values from four determinations made in two separate experiments. A control experiment (not shown here) was carried out under identical conditions except that ions were added at the end of the incubation period. In this circumstance, recoveries varied from 85 to 100% of control values. B, zero-time blank; C, control (in the presence of no competing ion).

1980*a*), although studies in guinea pig brain did not support this notion (Miller and Dawson, 1972). Studies that have examined base-exchange activity in microsomal subfractions of rat brain (Butler and Morell, 1983; Corazzi et al., 1986) have not resolved the question of whether or not this activity is due, at least in part, to an activity associated with contaminating neuronal plasma membranes (Goracci et al., 1973). Our data (Table 1), which indicate that all three base-exchange activities are enriched in SPMs prepared from rat brain at both saturating and subsaturating base concentrations, are in agreement with those of Miller and Dawson (1972) and Goracci et al. (1973) and may indicate that these enzymes play an essential role in excitable membranes. Ethanolamine and serine appear to be incorporated more actively than choline by this fraction (Table 1).

The possibility may still be entertained that the enrichment of base-exchange activity observed here in SPM is due to a 30% contamination of this fraction by smooth endoplasmic reticulum, as suggested by levels of the microsomal marker enzyme NADPH-cytochrome c reductase. Alternatively, the apparently high activity of NADPH-cytochrome c reductase in SPM may indicate that these membranes retain much of the synthetic machinery that is normally considered to be associated with endoplasmic reticulum. We have also measured substantial choline phosphotransferase activity in SPM (authors' unpublished data). In other studies, we have demonstrated that serine exchange is a component of pure axoplasm obtained from the squid giant axon, a finding suggesting that this activity is transported down the axon to nerve terminals (P. G. Holbrook and R. M. Gould, submitted).

In previous studies, no enrichment of base-exchange activity was observed either in synaptosomes (Porcellati et al., 1971; Buchanan and Kanfer, 1980*a*) or in SPM (Buchanan and Kanfer, 1980*a*). It should be pointed out that the procedures previously used for preparing synaptosomes and SPM involved more rigorous centrifugation conditions than those used for preparing microsomal membranes (Buchanan and Kanfer, 1980*a*) and that the process of resuspending and recentrifuging membranes can result in major losses of detectable base-exchange activity (Buchanan and Kanfer, 1980*b*). For this reason, we chose to prepare SPM by a technique (Jones and Matus, 1974) that is rapid and that involves relatively few centrifugation steps, separating SPM from other membranes by flotation rather than sedimentation. The enrichment of SPM base-exchange activity that we observed using this technique may be due to the fact that less activity was lost from this preparation or that perhaps an inhibitory factor was lacking or a stimulatory factor was present. The base-exchange activity that we measured in microsomes is lower than previously reported values (Butler and Morell, 1983; Hattori and Kanfer, 1985). This can be accounted for by the lower calcium concentrations and pH of our assay buffer.

Ethanolamine and serine exchanges have lower apparent K_m values (65 and 77 μM, respectively) than choline exchange (241 μM) in synaptosomes, and thus the former are probably more active at endogenous substrate concentrations. At the levels of free bases determined in microwaved rat brain (Table 3), choline and ethanolamine exchanges are not saturated with substrate; in contrast, serine exchange is saturated with, or perhaps even inhibited by, its substrate (see Table 2). These observations imply that the favored substrates in vivo are serine (which is present in brain at levels that would saturate the base-exchange enzymes) and ethanolamine (which, although not saturated, has the highest V_{max} for incorporation). Whole-brain levels of free choline are far below those needed for saturation, an observation suggesting that this pathway becomes significant only in loci where choline concentrations are markedly elevated, e.g., intrasynaptically, subsequent to the hydrolysis of released acetylcholine.

The kinetics for incorporation of the three bases into synaptosomal membranes (Table 2) are similar to those previously reported for rat brain microsomes (Kanfer, 1972). Free choline and/or ethanolamine can potentially be incorporated into synaptosomal PC and PE by base-exchange (1.27 ± 0.09 and 1.60 ± 0.17 nmol/mg of protein/h at V_{max}, respectively) less actively than by the Kennedy pathway (35.5 and 48.4 nmol/mg of protein/h, respectively) (Strosznajder et al., 1979) and more actively than by the stepwise methylation of PE (1.64 pmol/mg of protein/h) (Blusztajn and Wurtman, 1981).

Two choline uptake systems, which differ in their

affinities for choline, have been described in crude synaptosomal preparations of rat brain (Haga and Noda, 1973; Yamamura and Snyder, 1973; Wheeler, 1979) and in guinea pig intestine (Pert and Snyder, 1974): a high-affinity uptake system (K_m = 1.4–8 μM), which requires sodium, and a low-affinity system (K_m = 30–100 μM) which does not. The uptake systems in rat brain synaptosomes (Yamamura and Snyder, 1973) were reported to be unaffected by omission of calcium from the incubation medium, whereas calcium was necessary for uptake by guinea pig intestine (Pert and Snyder, 1974). Hence, one component of the low-affinity system could conceivably reflect base-exchange-mediated incorporation of choline into PC. Future studies of choline uptake should take this possibility into account, e.g., by analyzing the radioactive choline associated with phospholipid fractions.

Inhibition by inorganic polyvalent cations of calcium-dependent base-exchange reactions has been described in microsomal fractions of brain (Porcellati et al., 1971) and cardiac muscle (Filler and Weinhold, 1980). The presence of this phenomenon in synaptosomes is particularly interesting inasmuch as SPMs contain voltage-dependent calcium channels (Blaustein, 1975), which are blocked by inorganic polyvalent cations with a rank order of potency (Hagiwara and Byerly, 1981) similar to that observed here for the inhibition of synaptosomal base-exchange activity (Fig. 2). It has been suggested that phosphatidic acid, presumably an intermediate of calcium-stimulated base-exchange reactions, which also is formed subsequent to receptor-mediated hydrolysis of inositol phospholipids, may be involved in the receptor-mediated calcium gating mechanism (Putney et al., 1980; Salmon and Honeyman, 1980). It has also been suggested that phosphatidic acid may mediate calcium-dependent neurotransmitter release from depolarized synaptosomes (Harris et al., 1981). Consistent with the notion that the base-exchange reaction mechanism involves binding of calcium to phosphatidic acid is the fact that inorganic polyvalent cations block the lower-affinity choline exchange reaction more potently than the higher-affinity ethanolamine and serine exchange reactions (Fig. 2). These data thus raise the possibility that base-exchange is associated with presynaptic processes, like neurotransmitter release, thought to depend on the mobilization of calcium from the extracellular space.

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