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IONOPHORES X537A AND A23187

EFFECTS ON THE PERMEABILITY OF LIPID BIMOLECULAR MEMBRANES TO DOPAMINE AND CALCIUM

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SUMMARY

X537A carries dopamine across lipid bimolecular membranes. The rate of transport increases linearly with the X537A concentration and is independent of an electric field across the membrane. The evidence suggests that the permeating species is a neutral 1:1 complex between dopamine and X537A. A23187 does not transport dopamine. The permeability of the membrane to calcium increases as the square of the X537A concentration; the transport of calcium is also increased by A23187. With both ionophores, calcium is probably transported as an uncharged complex. Neither desmethylimipramine nor cocaine alters the transport of dopamine with X537A.

INTRODUCTION

Lipophilic ionophoric antibiotics increase the cation permeability of lipid membranes by forming a complex with cations and increasing their solubility in the lipid phase. They act as carriers for cations. Two carboxylic antibiotics, X537A and A23187, complex divalent cations [1, 2]. X537A, in addition, complexes amines, including the catecholamines dopamine, norepinephrine and epinephrine [1]. X537A and A23187 have been shown to act in many biological systems, including sarcoplasmic reticulum [3, 4], mitochondria [5], erythrocytes [6], salivary glands [7, 8], pancreas [9], neurohypophysis [10], platelets [11, 12] and mast cells [13–15], in a manner best explained by their actions as ionophores for calcium at membranes in these systems. In addition, X537A and A23187 both cause the release of norepinephrine from peripheral nerve-endings [16], and dopamine from synaptosomes [17]. X537A also causes the release of norepinephrine from chromaffin vesicles [18]. The release of catecholamines by A23187 is dependent on, and the release of catechol-

Abbreviation: DOPA, 3,4-dihydroxyphenyl-alanine.

amines by X537A independent of Ca^{2+} in the medium. It has been suggested that X537A acts as a membrane carrier for catecholamines [17–19]. This study investigates the effects of X537A and A23187 on the transport of dopamine and Ca^{2+} across lipid bimolecular membranes.

MATERIALS AND METHODS

Bimolecular membranes of electrical resistance $5 \cdot 10^6$–$5 \cdot 10^7$ ohm $\cdot$ cm^2 were formed according to the method of Mueller and Rudin [20] from two different mixtures of phosphatidylcholine (egg lecithin) and cholesterol (1:1 and 1:2 molar ratios) in *n*-decane, and from oxidized cholesterol dissolved in *n*-octane. The membrane was formed across a 1.37 mm diameter aperture in a teflon partition (100 μm thick) which was fixed between two plexiglass compartments, each containing 2 ml of an aqueous solution. The electrical properties of the membrane were measured by standard techniques [21] using calomel electrodes terminating in agar bridges (3 % agar in 0.1 M NaCl) in contact with the solutions. The membrane was visualized and the diameter measured with a reticule-containing microscope. 0.05 ml (2–20 μCi) of isotope ([^{14}C]dopamine hydrochloride, Spec. Act. 59 μCi/mol; $^{45}CaCl_2$, Spec. Act. 23.7 Ci/g Ca^{2+}; or L[^{14}C]DOPA Spec. Act. 37.6 μCi/mol) was introduced into the rear compartment. 0.05-ml samples were withdrawn periodically from the front compartment and their radioactivity measured in a scintillation counter. The permeability coefficient, P_d, was calculated from the rate of appearance of isotope in the front compartment according to the relationship $J = P_d A \Delta C$, where J is the flux of the radioactive species in mol/s; A, the membrane area in cm^2; and ΔC, the concentration difference across the membrane of the radioactive species in mol/cm^3 [21]. The P_d values measured in these experiments were usually less than 15 % of the unstirred layer-limited P_d (approx. $1 \cdot 10^{-3}$ cm/s), and unstirred layer corrections were made routinely [22].

RESULTS AND DISCUSSION

[^{14}C]Dopamine permeates the phosphatidylcholine/cholesterol and oxidized cholesterol bimolecular membranes in the presence, but not in the absence, of X537A (Fig. 1). The P_d for dopamine of phosphatidylcholine/cholesterol (1:1 and 1:2 molar ratios) membranes with 1 μM X537A in both compartments was 9.62 ± 1.02 (Mean$\pm$S.E.μ) $\times 10^{-5}$ cm/s in 17 experiments, and was independent of the buffer used (50, 100, 500 mM triethanolamine, 100 mM *N*-(2-acetamido)-2-iminodiacetic acid, and 5, 154 mM sodium phosphate). The P_d for dopamine increased linearly with the concentration of X537A, which suggests that dopamine is transported as a 1:1 complex with the ionophore (Fig. 1A, insert). The linear dependence was also observed with oxidized cholesterol membranes. Neither in the presence of an asymmetrical concentration of dopamine across the membrane (dopamine in the rear only, Fig. 1A) nor in the presence of a symmetrical concentration of dopamine across the membrane (Fig. 1B, after 50 min) did a potential difference across the membrane affect the rate of dopamine transport. In the latter case, if a charged species had been transported, assuming the Nernst-Planck flux equation, the flux would have changed 10-fold between +60 mV and −60 mV [23]. The absence of an

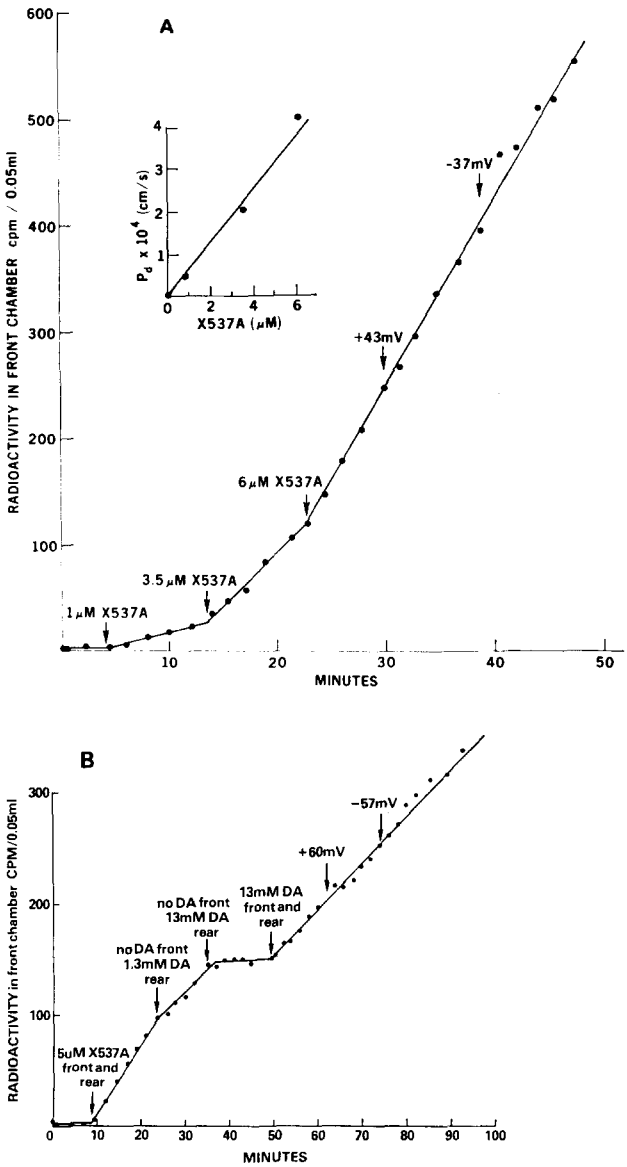

Fig. 1. (A) Effect of X537A concentration and electric field on the [^{14}C]dopamine transport across lipid bimolecular membranes. A bimolecular membrane was formed from a solution of phosphatidylcholine/cholesterol (1 : 2 molar ratio) in *n*-decane. The aqueous solution was 0.1 mM NaCl and 5 mM sodium phosphate (pH 7.2). At 0 min, [^{14}C]dopamine was added to the rear compartment; periodically thereafter, 0.05 ml samples were taken from the front. At the indicated times, X537A was added to the front and rear compartments. At 29 min, a constant potential of 43 mV (rear positive) was imposed across the membrane, and was replaced at 39 min by a constant potential of 37 mV (rear negative). The radioactivity in the rear compartment was $2.49 \cdot 10^5$ cpm per 0.05 ml; the membrane area, 0.0079 cm^2. The membrane conductance initially was $0.90 \cdot 10^{-8}$ Ω^{-1}, and rose linearly with X537A concentration to $7.5 \cdot 10^{-8}$ Ω^{-1} at 6.0 μM X537A. The insert shows the membrane permeability to [^{14}C]dopamine as a function of the X537A concentration. (B) The effects

effect of a potential difference on the flux implies that the transported species is not charged. X537A at phosphatidylcholine/cholesterol membranes caused a small conductance increase which was independent of dopamine, and at oxidized cholesterol membranes, no conductance increase. Since X537A increased the permeabilities of both membranes to [^{14}C]dopamine, the conductance effects of X537A do not correlate with the increases in [^{14}C]dopamine permeability. Because at pH 7.2 dopamine is positively charged and X537A is negatively charged, the 1:1 stoichiometry suggests a neutral complex whose movement across the membrane would not be affected by the presence of an electric field. If X537A carries dopamine across the membrane, and must then return in an electrically silent manner, X537A should return in complex with a cation. This possibility and the nature of the cation involved were investigated.

If the rate of return of X537A were limiting, then an increase in transportable cation in the front compartment should increase the flux of [^{14}C]dopamine from the rear. Dopamine is such a cation. When the rear compartment contained 50 μM [^{14}C]dopamine, addition of non-radioactive dopamine to the front caused a 50–100 % increase in [^{14}C]dopamine transport. It was found that the relative effect of non-radioactive dopamine added to the front on the rate of [^{14}C]dopamine transport from the rear to the front compartment could be augmented by first increasing the concentration of non-radioactive dopamine in the rear (Fig. 1B). Addition of non-radioactive dopamine (1.3 and 13 mM) to the rear compartment decreased the flux of [^{14}C]dopamine across the membrane. After the addition of 13 mM non-radioactive dopamine to the rear, the [^{14}C]dopamine flux fell to 5 % of its original value. The addition of 13 mM non-radioactive dopamine to the front restored the flux of [^{14}C]-dopamine to 65 % of its original value (Fig. 1B).

It appears that the addition of non-radioactive dopamine to the rear compartment increases the transport of the dopamine-X537A complex to the front suffi-

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of increasing concentrations of non-radioactive dopamine in each compartment and the effects of electric field on the X537A-induced transport of [^{14}C]dopamine through bimolecular membranes. An oxidized cholesterol bimolecular membrane was formed in 100 mM triethanolamine (pH 7.2). At 0 min, [^{14}C]dopamine (50 μM final concentration) was added to the rear compartment and 0.05 ml samples were taken periodically from the front. At 9 min, X537A was added to the front and rear compartments. Thereafter, non-radioactive dopamine (DA) was added to the front and rear compartments. The numbers represent final concentrations. The flux of [^{14}C]dopamine in the presence of X537A was initially 6.57 cpm/0.05 ml per min. From the specific activity of the dopamine in the rear and the volume of the sample from the front, the total unidirectional flux of dopamine was calculated to be $2.26 \cdot 10^{-12}$ mol/min. When the concentration of dopamine in the rear was raised to 1.3 mM with non-radioactive dopamine, the [^{14}C]dopamine flux fell to 4.17 cpm/0.05 ml per min, whereas the total unidirectional flux rose to $3.74 \cdot 10^{-11}$ mol/min. When the concentration of dopamine in the rear was further increased to 13 mM, the [^{14}C]dopamine flux fell to 0.36 cpm/0.05 ml per min while the total unidirectional flux remained approximately the same, $3.23 \cdot 10^{-11}$ mol/min. Upon addition of 13 mM non-radioactive dopamine to the front, the [^{14}C]dopamine flux rose to 4.28 cpm/0.05 ml per min and the total unidirectional flux rose to $3.83 \cdot 10^{-10}$ mol/min. The difference in electrical potential across the membrane between 0 and 62 min was 0. At 62 min, a constant potential difference of +60 mV (rear positive) was applied across the membrane and, at 74 min, replaced by a constant potential difference of −57 mV. The flux did not change in the presence of an electrical potential across the membrane. The radioactivity in the rear was $2.90 \cdot 10^5$ cpm per 0.05 ml, and the membrane area was 0.0087 cm^2. The membrane conductance was $< 5 \cdot 10^{-10}\ \Omega^{-1}$ throughout the experiment.

ciently to reduce the concentration of X537A at the rear interface. The return of X537A from the front now becomes rate limiting in the transport of [^{14}C]dopamine, and the [^{14}C]dopamine flux decreases. The addition of a transportable cation (dopamine$^+$) to the front permits the formation of a neutral complex with X537A and facilitates the return of X537A to the rear, thereby restoring the [^{14}C]dopamine flux. The existence of "accelerative exchange diffusion" [24] suggests that X537A functions as a mobile carrier in the membrane. Increasing the H^+ concentration in the front compartment by 3.5-fold (pH change from 7.20 to 6.65) increased the flux of [^{14}C] dopamine by 50–100 %, independent of the dopamine concentration in the rear (50 μM to 12.6 mM). In 50 mM triethanolamine, neither triethanolamine (3.5-fold increase) nor Na^+ (141 mM) when added to the front chamber affected the transport of [^{14}C]dopamine. Dopamine and H^+ added to the front compartment were specific, then, in increasing the transport of [^{14}C]dopamine.

The transport of dopamine by X537A is specific, as L-DOPA, differing from dopamine by the addition of a carboxyl group, was not transported. Dopamine does not penetrate the bimolecular membrane ($P_d < 5 \cdot 10^{-6}$ cm/s) in the presence of concentrations of A23187 as high as 6.3 μM. A23187, then, is not a carrier for dopamine through the bimolecular membrane.

Ca^{2+} permeates the phosphatidylcholine:cholesterol bimolecular membrane in the presence, but not in the absence, of the ionophores X537A and A23187 (Fig. 2). The P_d for $^{45}Ca^{2+}$ in the absence of ionophore was $< 5 \cdot 10^{-6}$ cm/s, The P_d for $^{45}Ca^{2+}$ with X537A increased with the square of the concentration of X537A (Fig. 2A, insert), which suggests that each molecule of Ca^{2+} is transported as a complex with two molecules of X537A. This is in contrast to the 1:1 stoichiometry observed in the transport of dopamine with X537A. X537A is a more potent carrier of dopamine than of Ca^{2+} at concentrations of the ionophore between 0 and 15 μM. The P_d for $^{45}Ca^{2+}$ varied as the A23187 concentration raised to the 1–1.7 power. This variability contrasts with the consistent dependency of $^{45}Ca^{2+}$ transport on the square of the X537A concentration, and may be related to reported ambiguities in the stoichiometry of Ca^{2+} binding to A23187 [25]. In our experiments, A23187 is a far more potent carrier of Ca^{2+} than is X537A (Fig. 2).

The small increases in conductance observed with X537A and A23187 are independent of the presence of Ca^{2+} ($5 \cdot 10^{-5}$ M). If Ca^{2+} is transported as a charged species, the Ca^{2+} flux can be estimated from the electrical conductance [26]. Even if the observed conductance increases were attributable to Ca^{2+}, they would account for, at most, only 10 % of the flux of Ca^{2+} observed with X537A, and 1 % of that observed with A23187 (Fig. 2).

Because the psychoactive drugs desmethylimipramine and cocaine inhibit the transport of biogenic amines across the plasma membranes of sympathetic nerve terminals, their effects on the X537A-mediated transport of [^{14}C]dopamine in bimolecular membranes was investigated. Neither desmethylimipramine ($3 \cdot 10^{-3}$ M) nor cocaine ($1 \cdot 10^{-5}$ M) in the rear compartment affected the rate of [^{14}C]dopamine transport with X537A.

The experiments on bimolecular membranes provide a physical model for the biological actions of X537A and A23187. In agreement with the bimolecular membrane results, the experiments in biological systems suggest that X537A, but not A23187, can act as a catecholamine ionophore [17–19], while both X537A and

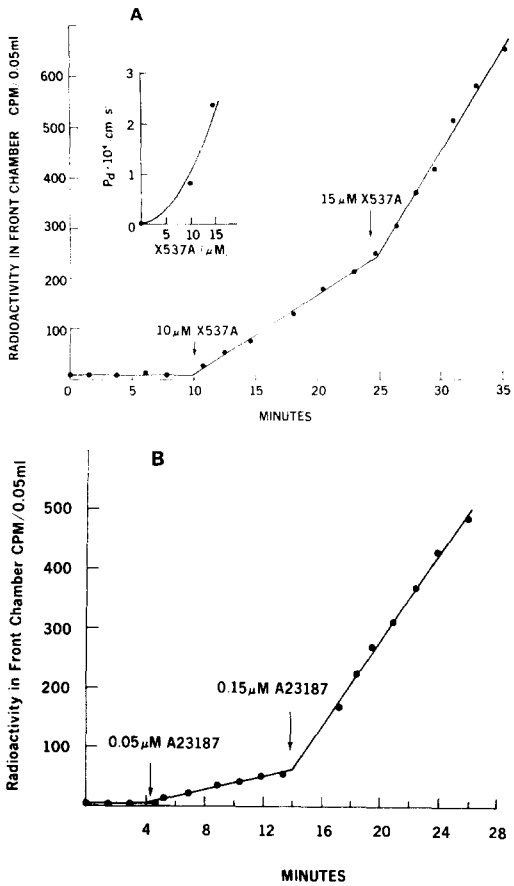

Fig. 2(A). The effect of X537A on the $^{45}Ca^{2+}$ permeability of a bimolecular membrane. A bimolecular membrane was formed from a solution of phosphatidylcholine/cholesterol (1 : 1 molar ratio) in *n*-decane. The aqueous solution was 100 mM triethanolamine and 50 μM $CaCl_2$ (pH7.5). At 0 time, $^{45}CaCl_2$ was added to the rear compartment; 0.05-ml samples were taken periodically from the front. At the indicated times, X537A was added to the front and rear compartments. The radioactivity in the rear compartment was $8.85 \cdot 10^5$ cpm/0.05 ml per min, and the membrane area was 0.0079 cm^2. The membrane conductance initially was $0.1 \cdot 10^{-8}\ \Omega^{-1}$ and rose linearly with X537A concentration to $3.2 \cdot 10^{-8}\ \Omega^{-1}$ at 15 μM X537A. The points in the insert show the membrane permeability of $^{45}Ca^{2+}$ as a function of the X537A concentration. The insert curve is drawn from a theoretical calculation of the increase in P_d as a function of the square of the X537A concentration. (B) The effect of A23187 on $^{45}Ca^{2+}$ permeability. The experiment is identical with the one in A, except that A23187 was added to the front and rear compartments as indicated. The radioactivity in the rear compartment was $7.58 \cdot 10^5$ cpm per 0.05 ml, and the membrane area was 0.0079 cm^2. The membrane conductance was initially $0.09 \cdot 10^{-9}\ \Omega^{-1}$ and rose linearly with A23187 concentration to $5.0 \cdot 10^{-9}\ \Omega^{-1}$ at 0.15 μM A23187. From the slopes of the lines and the above data, the permeability to $^{45}Ca^{2+}$ at 0.05 and 0.15 μM A23187 was calculated to be $1.21 \cdot 10^{-5}$ and $7.79 \cdot 10^{-5}$ cm/s, respectively.

A23187 can act as Ca^{2+} ionophores [3–17, 19]. As the transport of catecholamines and calcium through biological membranes may be mediated by naturally occurring ionophores, interactions between X537A or A23187 and lipid bimolecular membranes may also provide physical models for naturally occurring transport systems.

While this manuscript was in preparation, Celis et al. [27] and Schadt and Haeusler [28] showed that X537A caused a cation-dependent increase in the electrical conductance of lipid bimolecular membranes. Lower concentrations of cations (dopamine$^+$ or Ca^{2+}) and X537A in our studies gave large fluxes of dopamine and Ca^{2+} without accompanying dopamine- or Ca^{2+}-dependent electrical conductances. Extrapolation from our experiments suggests that the Ca^{2+} flux measured by these workers as Ca^{2+}-dependent conductance represents a small fraction of the total Ca^{2+} flux, the greater part of which is transported in an electrically silent manner. In addition, Schadt and Haeusler [28] showed, in agreement with our findings, that X537A transports catecholamines. They did not report electrical effects associated with catecholamine transport.

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