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'Slow' K^+-stimulated Ca^{2+} influx is mediated by Na^+–Ca^{2+} exchange: a pharmacological study

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K^+-stimulated $^{45}Ca^{2+}$ influx was measured in rat brain presynaptic nerve terminals that were predepolarized in a K^+-rich solution for 15 s prior to addition of $^{45}Ca^{2+}$. This 'slow' Ca^{2+} influx was compared to influx stimulated by Na^+ removal, presumably mediated by Na^+–Ca^{2+} exchange. The K^+-stimulated Ca^{2+} influx in predepolarized synaptosomes, and the Na^+-removal-dependent Ca^{2+} influx were both saturating functions of the external Ca^{2+} concentration; and both were half-saturated at 0.3 mM Ca^{2+}. Both were reduced about 50% by 20 μM Hg^{2+}, 20 μM Cu^{2+} or 0.45 mM Mn^{2+}. Neither the K^+-stimulated nor the Na^+-removal-dependent Ca^{2+} influx was inhibited by 1 μM Cd^{2+}, La^{3+} or Pb^{2+}, treatments that almost completely inhibited K^+-stimulated Ca^{2+} influx in synaptosomes that were not predepolarized. The relative permeabilities of K^+-stimulated Ca^{2+}, Sr^{2+} or Ba^{2+} influx in predepolarized synaptosomes (10:3:1) and the corresponding selectivity ratio for Na^+-removal-dependent divalent cation uptake (10:2:1) were similar. These results strongly suggest that the K^+-stimulated 'slow' Ca^{2+} influx in predepolarized synaptosomes and the Na^+-removal-dependent Ca^{2+} influx are mediated by a common mechanism, the Na^+–Ca^{2+} exchanger.

Introduction

Pinched-off presynaptic nerve terminals isolated from brain tissue, synaptosomes, have been extensively used to study the relationship between Ca^{2+} and neurosecretion (e.g. Refs. 1–3). When synaptosomes are depolarized in a K^+-rich solution containing a low concentration of Ca^{2+} (0.02 mM), two components of K^+-stimulated Ca^{2+} influx are seen [4]: a large, 'fast', transient component (approx. 0.2 nmol/mg protein per s) that is inhibited by low (under 1 μM) concentrations of Cd^{2+} and the lanthanides, and a smaller, 'slow', sustained component (approx. 0.04 mmol/mg protein per s) that is inhibited only at much higher Cd^{2+} and lanthanide concentrations [5]. The purpose of the investigations reported here was to examine the properties of the 'slow' component and to provide pharmacological evidence that this component is identical to Ca^{2+} uptake stimulated by Na^+ removal which is presumed to be mediated by Na^+–Ca^{2+} exchange.

The pharmacological properties of the 'fast' component of Ca^{2+} influx have been extensively investigated. This component is not sensitive to inhibition by the dihydropyridine Ca^{2+} antagonists [6,7] (but see Ref. 8), but it is inhibited by picomolar concentrations of the marine snail toxin, ω-conotoxin [9,10]. These characteristics, taken together with its sensitivity to inorganic blockers [5], make the 'fast' Ca^{2+} influx resemble the inward Ca^{2+} current that is carried by N-type Ca^{2+} channels recently described by Tsien and co-workers (Ref. 11; see reviews in Refs. 12, 13). The properties of the 'slow' component of Ca^{2+} influx, however, have not been so well studied.

Recently, it has been suggested that the 'slow' influx is mediated by a Na^+–Ca^{2+} exchanger operating in the 'reverse' mode, i.e., moving Ca^{2+} into the nerve terminals while extruding Na^+ [7,8,14,15]. This suggestion is based on the finding that this 'slow' influx component is reduced, or abolished, when synaptosomes are depleted of Na^+, while it is increased when synaptosomes are Na^+ loaded [14]. Nerve-terminal Ca^{2+} transport is also affected by changes in the external Na^+ concentration [16]. In addition, since Na^+–Ca^{2+} exchange is not electrically neutral, with three or more Na^+ ions exchanged for every Ca^{2+} ion [17–19], it might also move

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Abbreviations: DEEA, *N,N*-bis(2-[bis(carboxymethyl)amino]ethyl) glycine; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N'*-tetra-acetic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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Ca^{2+} into the nerve terminals during depolarization of the synaptosomes, even at high external Na^+ concentrations. Thus, this pathway could serve to carry Ca^{2+} into nerve terminals and thus affect neurotransmitter release during physiological activation.

To further characterize the pathway(s) that mediate the 'slow' component of K^+-stimulated Ca^{2+} influx, we examined its susceptibility to inhibition by several metal cations, and compared it with the susceptibility of Na^+-removal-dependent Ca^{2+} influx to inhibition by the same metal cations. This Na^+-removal-dependent Ca^{2+} influx is primarily mediated by a Na^+–Ca^{2+} exchanger [16,20,21]. We also examined the relative permeabilities of both pathways to Ca^{2+}, Sr^{2+} and Ba^{2+}. The results support the notion that the 'slow' component of K^+-stimulated Ca^{2+} influx is mediated by a Na^+–Ca^{2+} exchange mechanism.

Methods

Preparation of Synaptosomes

Synaptosomes were prepared from rat forebrains by the method of Nagy and Delgado-Escueta [22]. In brief, the P_2 synaptosomal pellet was resuspended in 0.25 M sucrose containing 5 mM Hepes (pH adjusted to 7.4 with Tris), 0.1 mM EDTA and 7% (v/v) Percoll. This suspension was layered onto a gradient consisting of 10 and 15% Percoll in 0.25 mM sucrose solution as above. This gradient was centrifuged at $15000 \times g$ for 30 min and the material from the 10%/15% interface was resuspended in Na^+ solution that contained (in mM): NaCl, 145; KCl, 5; $MgCl_2$, 3; glucose, 10; Hepes, 10; and DEEA (a general chelator of heavy metals), 0.001; the pH was adjusted to 7.4 with NaOH. The diluted suspension was centrifuged, and the pellet was resuspended in Na^+ solution with 0.02 mM Ca^{2+} added. This concentration was chosen to minimize Ca^{2+} overloading. The diluted synaptosome suspension was warmed at 30 °C for 20 min before proceeding with the experiments.

$^{45}Ca^{2+}$ loading and determination of $^{45}Ca^{2+}$ efflux

Aliquots of the synaptosome suspension, typically 20 μl, were ejected into tubes containing 20 μl Na^+ solution with Ca^{2+}, and 0.5 to 1 μCi of $^{45}Ca^{2+}$, to induce $^{45}Ca^{2+}$ loading (see below). The tubes with radioisotope were vigorously vortexed during the addition of the synaptosomes to ensure effective mixing. After at least 30 min, uptake of radiotracer was stopped by the addition of 4.5 ml ice-cold 0 Na^+ (Na^+ replaced isosmotically with *N*-methyl-D-glucamine) solution containing 1 mM EGTA and no added Ca^{2+} (pH adjusted to 7.4 with HCl; quench solution), followed by filtration (Schleicher and Schuell No. 25 glass fiber filters). The filters were immediately rinsed twice with additional 4.5 ml aliquots of quench solution. Radioactivity retained on the filters was determined by liquid scintillation spectrometry, and served as a measure of the Ca^{2+} load taken up by the synaptosomes. Efflux was determined by loading the synaptosomes with $^{45}Ca^{2+}$ as described above, then adding an aliquot of the loaded synaptosomes to 1 ml of choline$^+$/Na^+ solution (5–145 mM Na^+; 5 mM K^+). Efflux of $^{45}Ca^{2+}$ was allowed to proceed for 10 s, at which time the solutions were quenched, rapidly filtered and the filters rinsed twice with quench solution as described above. For K^+-stimulated $^{45}Ca^{2+}$ efflux, synaptosomes were $^{45}Ca^{2+}$ loaded as described above then were predepolarized for 15 s in 77.5 K^+ + 72.5 mM Na^+ with $^{45}Ca^{2+}$, before being added to solutions containing different K^+ concentrations (72.5 mM Na^+; varying choline) and unlabelled $^{40}Ca^{2+}$. Efflux was terminated after 10 s. The amount of $^{45}Ca^{2+}$ retained by the synaptosomes minus the initial Ca^{2+} load, was taken as a measure of efflux [16].

Determination of $^{45}Ca^{2+}$ influx

In experiments designed to measure Na^+-removal-dependent $^{45}Ca^{2+}$ influx, 20–25 μl aliquots of the synaptosome suspension were added to 1–25 ml of Na^+ solution containing 5–145 mM Na^+ (5 mM K^+) and 1 μCi/ml $^{45}Ca^{2+}$. At various intervals, ranging between 1 and 20 s, the suspensions were quenched, filtered, and the filters were rinsed with two additional aliquots of quench solution as described above. The $^{45}Ca^{2+}$ retained by the filters was then determined. Na^+-removal-dependent Ca^{2+} influx was defined as the $^{45}Ca^{2+}$ influx in the Na^+ solution, at various Na^+ concentrations, minus the $^{45}Ca^{2+}$ influx in normal (145 mM) external Na^+ [16].

In experiments designed to measure K^+-stimulated 'slow' Ca^{2+} influx, 20–25 μl aliquots of synaptosomes were added to 20–25 μl of a K^+-rich solution (77.5 mM K^+; 72.5 mM Na^+), and 0.02 mM unlabelled $^{40}Ca^{2+}$, for 'predepolarization' [23]. After 15 s, a large excess of various K^+-rich solutions (72.5 mM Na^+) containing $^{45}Ca^{2+}$ were added, and influx was measured over 1–20 s as above. 'Slow' Ca^{2+} influx was defined as the $^{45}Ca^{2+}$ influx in predepolarized synaptosomes minus the $^{45}Ca^{2+}$ influx in normal Na^+ solution.

For inhibitor studies, $^{45}Ca^{2+}$ influx was stimulated by 36 mM Na^+ (5 mM K^+) or by 57.5 mM K^+ (72.5 mM Na^+), concentrations which were deemed to produce quantitatively similar amounts of Ca^{2+} uptake. For these studies, DEEA was left out of all solutions. Various concentrations of the inorganic cations were added at time zero and uptake was terminated after 10 s, as described above. Uptake was plotted according to the equation:

$$\% \text{ relative } Ca^{2+} \text{ uptake} = 100\%/(1 + [I]/K_1)$$

where I is the inhibitor and K_I is the concentration producing half-maximal block.

All samples were done in replicates of 3-5. Results are given as means ± S.E. In each experiment, samples were taken from the synaptosome suspension for protein determination [24], and tissue blanks were determined by adding the synaptosome suspension to quench solutions containing an aliquot of $^{45}Ca^{2+}$. The tissue-blank values were subtracted from the reported results.

Results

When synaptosomes were depolarized in a K^+-rich solution (77.5 mM K^+/72.5 mM Na^+), there was an increase in Ca^{2+} influx. This K^+-dependent Ca^{2+} uptake (influx in K^+-rich solution minus influx in low K^+ solution; Fig. 1A; solid symbols) had a complicated time-course: as previously reported, there was an initial burst of Ca^{2+} accumulation, followed by a slower, prolonged component of Ca^{2+} influx [23]. The remaining experiments in Fig. 1 show how these two components can be distinguished. The 'slow' component was eliminated when synaptosomes were preincubated in Na^+-free solution (Fig. 1A; open symbols). The 'fast' component appears to be mediated by calcium channels that undergo inactivation [25], and could be eliminated by predepolarization, i.e., by preincubating synaptosomes in K^+-rich, depolarizing solution, before addition of $^{45}Ca^{2+}$ (Fig. 1B; solid symbols). When synaptosomes were subjected to both resuspension in Na^+-free solution and predepolarization. K^+-stimulated Ca^{2+} influx

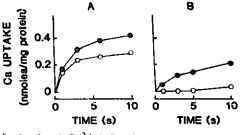
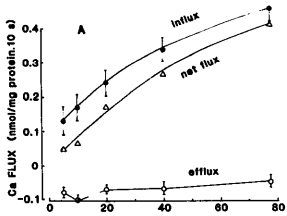


Fig. 1. K^+-stimulated Ca^{2+} influx in synaptosomes preincubated in normal Na^+ (145 mM; solid circles) or in zero Na^+ (145 mM choline; open circles) solutions with 0.02 mM Ca^{2+}. A. Total Ca^{2+} influx. Aliquots of the synaptosome suspensions were added to K^+-rich solutions (77.5 mM K^+/72.5 mM Na^+ or 77.5 mM K^+/72.5 mM choline$^+$) containing $^{45}Ca^{2+}$. Influx was terminated after 1, 3, 5 and 10 s. B. Predepolarization. Aliquots of the synaptosome suspension were added to K^+-rich solutions (as above) with 0.02 mM unlabelled $^{40}Ca^{2+}$ for 15 s for predepolarization. At 15 s, tracer $^{45}Ca^{2+}$ was added and influx continued for the indicated times. The control influx of $^{45}Ca^{2+}$ in normal (145 mM) Na^+ solution (amounting to approx. 0.06 nmol/mg protein at 1 s and to 0.15 nmol/mg protein at 10 s) has been subtracted from all the values shown. Standard error bars have been omitted but were about 15% in the different experiments.

was almost totally eliminated (Fig. 1B; open symbols). In the remainder of this article, unless otherwise specified, the term K^+-dependent Ca^{2+} influx refers to the 'slow' component of Ca^{2+} influx measured following predepolarization.

The effect of depolarization, produced by increasing the external K^+ concentration, on Ca^{2+} fluxes in predepolarized synaptosomes is illustrated in Fig. 2A. Synaptosomes that had been resuspended in Na^+ solution were predepolarized by 15 s incubation in a K^+-rich solution (77.5 mM K^+/72.5 mM Na^+) containing 0.02



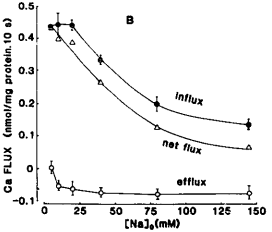


Fig. 2. The effects of changing $[Na]_o$ and $[K]_o$ in Ca^{2+} fluxes. A. Effect of varying $[K]_o$ in predepolarized synaptosomes. For influx measurements (●), synaptosomes were predepolarized for 15 s in 77.5 mM K^+ + 72.5 mM Na^+ with unlabelled $^{40}Ca^{2+}$ before addition to solutions containing $^{45}Ca^{2+}$ and different $[K]_o$; influx was terminated after 10 s. For efflux measurements, synaptosomes were equilibrated in normal Na^+ solution containing 0.02 mM $^{45}Ca^{2+}$ for at least 30 min. After isotopic equilibration, aliquots of the suspension were predepolarized for 15 s in 77.5 mM K^+ + 72.5 mM Na^+ with $^{45}Ca^{2+}$ before addition to solutions containing different $[K]_o$ and unlabelled $^{40}Ca^{2+}$. Efflux (○) was terminated after 10 s. Net flux (△) was calculated as influx plus efflux. B. Effect of varying $[Na]_o$ on Ca^{2+} fluxes. For influx measurements (●) synaptosomes were added to media containing the Na^+ concentrations shown on the abscissa and 0.02 mM $^{45}Ca^{2+}$. Uptake was terminated after 10 s. For efflux measurements, synaptosomes were equilibrated in a normal Na^+ solution containing 0.02 mM $^{45}Ca^{2+}$ for at least 30 min. After isotopic equilibration, aliquots of the suspension were diluted into large volumes of media containing different Na^+ concentrations and unlabelled $^{40}Ca^{2+}$. Efflux (○) was terminated after 10 s. Net flux (△) was calculated as uptake plus efflux (see Ref. 16).

mM (unlabelled) $^{40}Ca^{2+}$; the suspension was then added to a solution containing 0.02 mM $^{45}Ca^{2+}$ and varying amounts of K^+ (Na^+ kept constant at 72.5 mM), and Ca^{2+} influx was measured (solid circles). In parallel experiments, synaptosomes that had been resuspended in Na^+ solution with $^{45}Ca^{2+}$ were predepolarized in a K^+-rich solution containing $^{45}Ca^{2+}$ subsequently, the suspension was diluted into a large volume of a solution containing $^{40}Ca^{2+}$ and different concentrations of K^+, and the $^{45}Ca^{2+}$ remaining after 10 s was used to determine Ca^{2+} efflux (open symbols). The net Ca^{2+} flux (triangles) increased with increasing K^+ to approx. 0.4 nmol of Ca^{2+}/mg protein at $[K^+]_0 = 77.5$ mM. These results indicate that the 'slow' component of Ca^{2+} flux is voltage-dependent or K^+-dependent or both.

Fig. 2B shows the effect (solid circles) of decreasing the external Na^+ concentration on Ca^{2+} fluxes. The net Ca^{2+} flux (triangles) increased to 0.4 nmol of Ca^{2+}/mg protein over 10 s, as the external Na^+ concentration was reduced from 145 to 5 mM (K^+ kept constant at 5 mM). This is the $[Na^+]_0$-dependent Ca^{2+} flux.

In order to demonstrate that the 'slow' phase of depolarization-induced Ca^{2+} influx and the Na^+-removal-induced Ca^{2+} influx are mediated by the same mechanism, it was helpful to select experimental conditions where the Ca^{2+} influx in either situation was approximately the same. From the results in Fig. 2, values of 57.5 mM K^+ (72.5 mM Na^+) and 36 mM Na^+ (5 mM K^+), respectively, were chosen. The time-course of $^{45}Ca^{2+}$ influx in predepolarized synaptosomes incubated in a solution containing 57.5 mM K^+ and $^{45}Ca^{2+}$ influx in a solution containing 36 mM Na^+ were indistinguishable (results not shown). In most subsequent experiments, therefore, Ca^{2+} influx under these two conditions was compared. It must be emphasized that in the case of K^+-stimulated 'slow' influx, the effect of depolarization by K^+ is being studied on top of the effect of lowering Na^+ to 72.5 mM, which by itself stimulates a net Ca^{2+} influx (Fig. 2).

Fig. 3 illustrates the effect of increasing the external Ca^{2+} concentrations on both the Na^+ removal- and the K^+-dependent Ca^{2+} influxes. For both series of experiments, the results could be described by the same saturation curve, with a half-maximal flux at 0.3 mM. This result would be expected if both the Na^+ removal- and the K^+-dependent Ca^{2+} influxes were mediated by the same mechanism. The half-saturation value is similar to that obtained in an earlier study of the 'slow' component of K^+-stimulated Ca^{2+} uptake of 0.4 mM [26]. The saturation seen is not due to calcium overloading of the synaptosomes since uptake continues to increase in 2 mM Ca^{2+} for up to 96 s (Ref. 27, Fig. 2).

Hg^{2+} and Cu^{2+} are divalent cations that preferentially inhibit the 'slow' component, whereas Mn^{2+} is a selective inhibitor of the 'fast' component of Ca^{2+}

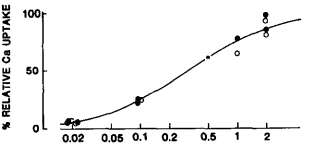


Fig. 3. Effect of varying $[Ca]_0$ on $^{45}Ca^{2+}$ influx. Synaptosomes were incubated for 10 s in media containing either 36 mM Na^+ (○) or 57.5 mM K^+ (after predepolarization; ●) with various concentrations of Ca^{2+}. $^{45}Ca^{2+}$ taken up in normal (145 mM) Na^+ at the various Ca^{2+} concentrations have been subtracted. The results of three different experiments have been combined and normalized to the uptake measured with 0.5 mM Ca^{2+} (*). The solid line through the points has been drawn according to the equation:

$$\% \text{ relative } Ca^{2+} \text{ uptake} = 100\%(1 + K_{Ca}/[Ca]_0)$$

where K_{Ca} had a value of 0.3 mM.

influx [5]. The inhibitory effects of Hg^{2+}, Cu^{2+} and Mn^{2+} on the K^+-dependent 'slow' Ca^{2+} influx and on Na^+-removal-dependent Ca^{2+} influx were studied. Both modes of Ca^{2+} influx were inhibited with similar K_I values. K_I values for Hg^{2+}, Cu^{2+} and Mn^{2+} were 20, 20 and 450 μM, respectively, for both modes of Ca^{2+} influx.

The cations Cd^{2+}, La^{2+} and Pb^{2+}, which at a concentration of 1 μM effectively inhibit the 'fast' component of Ca^{2+} influx (K^+-stimulated $^{45}Ca^{2+}$ uptake measured at 1 s), have much smaller effects on either Na^+-removal-dependent and 'slow' K^+-dependent Ca^{2+} influxes (Fig. 4).

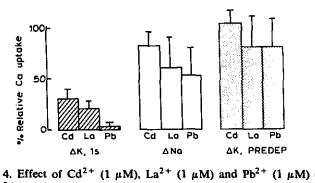


Fig. 4. Effect of Cd^{2+} (1 μM), La^{2+} (1 μM) and Pb^{2+} (1 μM) on $^{45}Ca^{2+}$ influx. Hatched bars (ΔK, 1 s): synaptosomes were incubated for 1 s in a solution containing 77.5 mM K^+ and 72.5 mM Na^+, and 0.02 mM $^{45}Ca^{2+}$. Open bars (ΔNa): synaptosomes were incubated in low Na^+ solution (5 mM Na^+/140 mM choline/5 mM K^+) and 0.02 mM $^{45}Ca^{2+}$ for 10 s. Dotted bars (ΔK, predep): synaptosomes were predepolarized in a solution containing 77.5 mM K^+ and 72.5 mM Na^+, and 0.02 mM cold $^{40}Ca^{2+}$. After 15 s, $^{45}Ca^{2+}$ was added and the incubation continued for 10 s. The $^{45}Ca^{2+}$ taken up in normal Na^+ solution (145 mM Na^+), containing the appropriate inhibitory ion, has been subtracted. Results are expressed as percentage of $^{45}Ca^{2+}$ taken up in the absence of inhibitor for all three conditions.

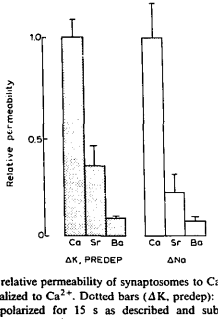


Fig. 5. The relative permeability of synaptosomes to Ca^{2+}, Sr^{2+} and Ba^{2+} normalized to Ca^{2+}. Dotted bars (ΔK, predep): synaptosomes were predepolarized for 15 s as described and subsequently, an aliquot was added to K^+-rich (57.5 mM K^+) solution containing 0.02 mM $^{45}Ca^{2+}$, 0.02 mM $^{85}Sr^{2+}$ and 0.02 mM $^{133}Ba^{2+}$. Uptake was terminated after 10 s. Open bars (ΔNa): synaptosomes were added to low Na^+ solution (36 mM Na^+/119 mM choline/5 mM K^+) containing 0.02 mM $^{45}Ca^{2+}$, 0.02 mM $^{85}Sr^{2+}$ and 0.02 mM $^{133}Ba^{2+}$. Uptake was terminated after 10 s. The permeability (P) of a divalent cation, M^{2+}, relative to the permeability of Ca^{2+} is given by the equation:

$$P_{M^{2+}}/P_{Ca^{2+}} = (J_{M^{2+}}/J_{Ca^{2+}}) \cdot ([Ca^{2+}]_0/[M^{2+}]_0)$$

where J is the influx of the radioisotope [26].

The divalent cations Sr^{2+} and Ba^{2+} can substitute for Ca^{2+} in many transport systems. The extent to which these cations could be taken up in synaptosomes via either the Na^+-removal-dependent or the K^+-dependent modes was determined. Synaptosomes were resuspended in a solution containing (mM) 0.02 Ca^{2+}, 0.02 Sr^{2+} and 0.02 Ba^{2+}, and the Na^+-removal- or 'slow' K^+-dependent uptake of $^{45}Ca^{2+}$, $^{85}Sr^{2+}$ and $^{133}Ba^{2+}$ was measured. The relative permeabilities of the synaptosomes for the different cations are given directly by the radiotracer flux ratios [26]. For the K^+-dependent uptake, the Ca/Sr/Ba ratio was 10 : 3 : 1, and for Na^+-removal-dependent uptake, the ratio was 10 : 2 : 1 (Fig. 5).

Discussion

The results presented here demonstrate that both the 'slow' component of K^+-stimulated Ca^{2+} influx and the Na^+-removal-dependent Ca^{2+} influx show the same saturation to the external Ca^{2+} concentration, and both have similar permeabilities to the alkali earth cations Ca^{2+}, Sr^{2+} and Ba^{2+}. Furthermore, both pathways are inhibited by similar concentrations of Hg^{2+}, Cu^{2+} and Mn^{2+} and both are relatively insensitive to inhibition by Cd^{2+}, La^{3+} and Pb^{2+}. Based on these pharmacological comparisons (see also Ref. 28), it is very likely (but not definitely established) that the two modes of Ca^{2+} influx share the same molecular machinery, Na^+–Ca^{2+} exchange.

In order to confirm this point, it must be shown that 'slow' Ca^{2+} influx is sensitive to changes in internal Na^+ concentration with Na^+-selective ionophores or by pretreatment in Na^+-free solutions (see Refs. 7, 14 and Fig. 1), but it has not been possible to quantify these changes. But with the advent of new methods for Na^+ measurement [29] and fluorescent dyes that can report intracellular Na^+ concentrations [30], such quantitative studies should soon be possible.

One possible problem with our studies is the use of K^+ concentration changes to alter the membrane voltage: it is possible that K^+ might have direct effects on the exchanger. However, investigations of Na^+–Ca^{2+} exchange in the squid giant axon, which have revealed much about its basic mechanisms (for reviews, see Refs. 31, 32), help to clarify this issue. Studies by Allen and Baker [33,34] where internal and external solutions were varied and where the axonal membrane was voltage clamped showed that K^+ did not have much of an effect on its own other than through membrane depolarization. If these results can be applied to vertebrate neurons, it follows that our results demonstrate voltage-dependence rather than a K^+-dependence of the Na^+–Ca^{2+} exchanger in rat brain nerve terminals. This voltage-dependence means that under conditions of depolarization, Na^+–Ca^{2+} exchange can operate in reverse and contribute a net inward Ca^{2+} flux.

The magnitude of this 'slow' Ca^{2+} influx is 10–20% of the total Ca^{2+} influx that can be carried by presynaptic Ca^{2+} channels. However, it can be sustained for several minutes. In heart, Na^+–Ca^{2+} exchange operating in the reverse mode probably contributes Ca^{2+} to contraction (see, for example, Ref. 35). It is possible that such a mechanism may also contribute Ca^{2+} to transmitter release from neurons. Since neurotransmitter release increases as a steep power function of the internal Ca^{2+} concentration [3,36], the extra calcium influx mediated by Na^+–Ca^{2+} exchange might have important physiological consequences.

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