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Tricyclic antidepressant inhibition of depolarization-induced uptake of calcium by synaptosomes from rat brain

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Release of neurotransmitters from nerve terminals is dependent upon an influx of extracellular calcium that is mediated by voltage-gated channels [1]. Depolarization-induced calcium uptake can be studied in synaptosomes isolated from mammalian brain using $^{45}Ca^{2+}$ as a radiotracer and elevated K^+ concentrations to decrease resting membrane potential [2]. This uptake is rapid (<1 min), saturable, and susceptible to inhibition by several divalent ions, including Co^{2+}, Mn^{2+} and Cd^{2+}, which have been shown to block Ca^{2+} currents in electrophysiological studies [3]. A number of psychoactive drugs interfere with synaptosomal calcium accumulation, including ethanol [4], barbiturates [5], neuroleptics [6], and benzodiazepines [7]. In the present paper, we report the inhibition by tricyclic antidepressant drugs of K^+-stimulated $^{45}Ca^{2+}$ uptake by rat brain synaptosomes.

Methods. Two buffered salt solutions were used: S buffer contained 130 mM NaCl, 5 mM KCl, 1.2 mM Na_2HPO_4, 0.1 mM $CaCl_2$, 10 mM glucose, and 20 mM Tris–Cl, pH 7.4. In K buffer, the NaCl was omitted and the KCl concentration was raised to 135 mM.

Synaptosomes were prepared by homogenizing the forebrains (telencephalon and diencephalon with the olfactory lobes removed) of 200–250 g male Wistar rats in 10 vol. of ice-cold 0.32 M sucrose. After centrifuging at 1000 *g* for 5 min, the supernatant fraction was centrifuged at 35,000 *g* for 25 min and the pellet was resuspended in 0.32 M sucrose. This crude synaptosomal-mitochondrial fraction was layered over a density gradient comprised of 15 ml of 0.8 M sucrose and 15 ml of 1.2 M sucrose and spun at 100,000 *g* for 1 hr in a swinging bucket rotor. The synaptosomes were recovered from the 0.8 to 1.2 M sucrose interface, slowly diluted with S buffer, spun at 30,000 *g* for 20 min and resuspended in S buffer at a concentration of 0.4 to 0.5 mg synaptosomal protein/ml. This final suspension was allowed to sit at room temperature for 60 min before uptake measurements were performed.

To measure calcium uptake, 500 μl of S buffer (for resting uptake) or K buffer (for stimulated uptake) containing 0.1 μCi $^{45}Ca^{2+}$ (New England Nuclear) was added to synaptosomes (0.2 to 0.3 mg protein) suspended in 500 μl S buffer. After an appropriate time (generally 20 sec), the samples were filtered through Whatman GF/B glass fiber filters. The filters were washed once with 2 ml of S buffer and their radioactivity content was determined by liquid scintillation counting. All uptake values were determined in triplicate. To assess the influence of various drugs on calcium uptake, the synaptosomes were preincubated with the drugs for 15 min in S buffer before the $^{45}Ca^{2+}$-containing influx medium was added.

Results and discussion. Dilution of synaptosomes with medium containing depolarizing concentrations of K^+ approximately doubled $^{45}Ca^{2+}$ uptake. This uptake reached 50% of the final level in about 15 sec and was 90% complete within 1 min. After 2 min, the calcium content of depolarized synaptosomes slowly decreased so that after 45 min the $^{45}Ca^{2+}$ content of synaptosomes diluted with S or K uptake medium was the same (data not shown). K^+-stimulated Ca^{2+} uptake was inhibited 50% by 3 mM Mn^{2+} and 0.28 mM Co^{2+} (not shown). The extent of $^{45}Ca^{2+}$ uptake at 2 min increased with K^+ concentration up to about 60 mM K^+. K^+-stimulated uptake was linearly related to tissue concentration up to at least 1 mg synaptosomal protein/ml.

The initial rate of K^+-stimulated Ca^{2+} uptake was estimated by measuring $^{45}Ca^{2+}$ uptake at 5 sec, at which time calcium uptake was 15–20% of the level reached by 1 min. A Lineweaver–Burk plot of this data was linear (Fig. 1) and indicated a maximum rate of uptake of 0.30 nmole $Ca^{2+} \cdot (\text{mg protein})^{-1} \cdot \text{sec}^{-1}$ and an apparent K_m of 0.37 mM Ca^{2+}.

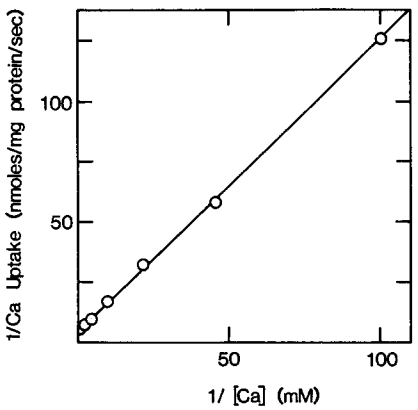

Fig. 1. Lineweaver–Burk plot of calcium uptake by rat brain synaptosomes. The rate of calcium uptake was estimated from the uptake of $^{45}Ca^{2+}$ 5 sec after abrupt dilution of synaptosomes into a medium containing 67.5 mM KCl. Calcium uptake was measured in the presence of ten concentrations from 0.01 to 10 mM (three data points at high Ca^{2+} concentrations are omitted from the plot for reasons of clarity). The line is drawn according to linear regression analysis of all the data, which indicates a maximum rate of uptake of 0.30 nmoles·(mg protein)$^{-1}$·sec^{-1} and a K_m of 0.37 mM. Each point is the average from three separate experiments.

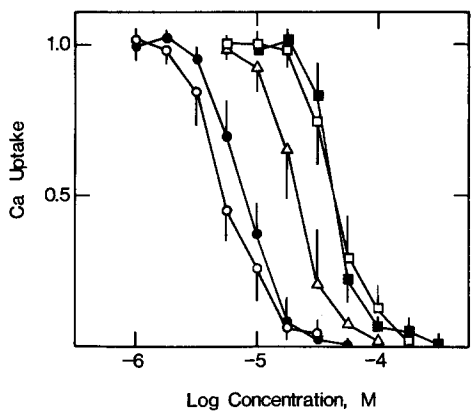

Fig. 2. K^+-stimulated Ca^{2+} uptake by rat brain synaptosomes in the presence of various tricyclic antidepressants. $^{45}Ca^{2+}$ uptake was measured 20 sec after dilution of the synaptosomes into influx medium containing $^{45}Ca^{2+}$ and either 5 or 67.5 mM K^+. Stimulated uptake was the difference between calcium uptake in the high and low K^+ conditions. The synaptosomes were preincubated for 15 min with the indicated concentrations of chlorimipramine (○), desmethylchlorimipramine (●), imipramine (□), desmethylimipramine (■) or amitriptyline (△). The points and bars indicate the means and standard deviations from three separate determinations.

Tricyclic antidepressant drugs inhibited K^+-stimulated Ca^{2+} uptake by rat brain synaptosomes (Fig. 2; Table 1). The IC_{50} values ranged from 5 to 50 μM, which is the same range as Ca^{2+} uptake inhibition by phenothiazenes and other neuroleptic drugs [6, 8]. Multiple components to K^+-stimulated Ca^{2+} uptake by nerve endings have been reported (e.g. Ref 2). A fast component of K^+-stimulated Ca^{2+} uptake lasts 2 sec, is inactivated by predepolarization of the membrane, and is inhibited by low concentrations of La^{3+} [2]. Slow calcium entry is much less sensitive to La^{3+} and is not affected by predepolarization. Both components of calcium entry have the same dependence on K^+ concentration and are equally sensitive to nickel, manganese and magnesium [2]. In the present study, tricyclic antidepressant drugs were equally effective at inhibiting K^+-stimulated Ca^{2+} uptake at 5 sec as at 20 sec. Thus, these drugs do not appear to have a selective effect on the fast phase of K^+-stimulated Ca^{2+} uptake.

Plasma concentrations of tricyclic antidepressants during therapeutic administration range from 10 to 100 ng/ml [9]. Tricyclic antidepressants accumulate in the brain, and under steady-state conditions a brain/plasma ratio of about 20 has been reported [9]. Since up to 95% of these drugs are bound to membranes and proteins, their effective concentrations at the synapse are difficult to estimate. The concentrations of tricyclic antidepressants which inhibited K^+-stimulated Ca^{2+} uptake by synaptosomes may be slightly higher than those attained during therapy. This may reflect the extreme nature of the depolarizing stimulus (dilution of synaptosomes into high potassium solutions). It should be noted, however, that tricyclic antidepressants are among the most potent blockers of K^+-stimulated Ca^{2+} uptake that have been reported.

A chloride substitution at the 3 position increased Ca^{2+} uptake inhibition 6- to 8-fold. Equivalent levels of inhibition were obtained by related tricyclic antidepressant drugs which were secondary and tertiary compounds (i.e. imipramine and desmethylimipramine, chlorimipramine and desmethylchlorimipramine, amitriptyline and nortriptyline). There is a similar lack of selectivity in tricyclic antidepressant interactions with the ion channel that is part of the nicotinic acetylcholine receptor [10, 11]. In contrast, norepinephrine uptake inhibition and psychomotor activation are more likely to be produced by tricyclic antidepressants which are secondary amines than the related tertiary amines, and the opposite sensitivity is associated with tricyclic antidepressant interaction with histamine (H_1 and H_2), alpha-adrenergic and muscarinic receptors, as well as the serotonin uptake system [1].

Table 1. Tricyclic antidepressant inhibition of K^+-stimulated $^{45}Ca^{2+}$ uptake by rat brain synaptosomes

Drug	IC_{50}* (μM)
Imipramine	43.9 ± 5.2
Desmethylimipramine	44.3 ± 10.4
Chlorimipramine	5.2 ± 2.6
Desmethylchlorimipramine	7.9 ± 2.1
Amitriptyline	22.6 ± 8.4
Nortriptyline	15.3 ± 7.6
Protriptyline	26.0 ± 5.2
Doxepin	13.9 ± 6.0

* Concentration of antidepressant which inhibited K^+-stimulated $^{45}Ca^{2+}$ uptake by 50%. The means and standard deviations from three experiments performed on different synaptosome preparations are listed.

K^+-stimulated Ca^{2+} uptake was not affected by a wide range of compounds which interact with biogenic amine receptors in the brain (Table 2). In addition, drugs which inhibit transport through voltage- and chemically-gated ion channels in excitable membranes did not interfere with Ca^{2+} uptake by synaptosomes (Table 2). This includes drugs which inhibit voltage-gated Ca^{2+} (verapamil and nifedipine) and K^+ (tetraethylammonium, phencyclidine) channels and the acetylcholine-gated channel that is part of nicotinic receptors (histrionicotoxins, phencyclidine, gephyrotoxin, pumiliotoxins and benzomorphans). Ion channels assume several conformational states (e.g. resting, activated, inactivated) which may differ in their interactions with drugs.

Table 2. Compounds which did not inhibit resting or K^+-stimulated $^{45}Ca^{2+}$ uptake by rat brain synaptosomes

Less than 10% inhibition at 100 μM:
Neurotransmitter receptor agonists and antagonists:
Acetylcholine, dopamine, serotonin, γ-aminobutyric acid, glycine, glutamate, norepinephrine, epinephrine, nicotine, atropine, *d*-tubocurarine, methylscopolamine, propranolol
Nicotinic receptor ion channel blockers:
Phencyclidine, phencyclidine methiodide, histrionicotoxin, histrionicotoxin methiodide, gephyrotoxin, pumiliotoxin A, pumiliotoxin C_I, pumiliotoxin C_{II}, *N*-allylnormetazocine, cyclazocine, pentazocine
Calcium channel blockers:
Verapamil, nifedepine

Less than 10% inhibition at 1 mM:
Valproic acid
Potassium channel blockers:
Tetraethylammonium, 4-aminopyridine, 2,6-diaminopyridine

In the present experiments, drugs were preincubated with polarized synaptosomes, presumably containing voltage-gated calcium channels in a resting configuration. Drugs which interact primarily with inactivated (densensitized) or activated forms of the channel might show little activity in the present assay. Therefore, the present characterization of drug interactions with the structures subserving K^+-stimulated Ca^{2+} influx in synaptosomes should not be considered exhaustive.

Tricyclic antidepressant drugs interfere with a number of neurochemical processes, including neuronal uptake of catechol and indole amines [12–14] and binding by several neurotransmitter receptors [15–17]. Therapeutic efficacy is not closely related to inhibition of any these processes, and it is likely that a spectrum of actions underlie their behavioral effects. The present results raise the possibility that inhibition of depolarization-induced Ca^{2+} uptake by nerve terminals contributes to the clinical response. It should be noted, however, that a number of known blockers of K^+-stimulated Ca^{2+} uptake by synaptosomes, notably neuroleptic drugs [6], are devoid of antidepressant activity.

In summary, tricyclic antidepressant drugs blocked K^+-stimulated Ca^{2+} uptake by rat brain synaptosomes with IC_{50} values in the range of 5–50 μM. There was no selectivity for related drugs which were secondary versus tertiary amines. A number of drugs which blocked voltage-gated potassium and calcium channels, as well as the chemically-gated ion channel associated with the nicotinic acetylcholine receptor, did not inhibit calcium uptake by synaptosomes. It is possible that this action contributes to the therapeutic effectiveness of tricyclic antidepressant drugs.

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