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Short communication

Inhibition of K^+-induced Ca^{2+} uptake in rat hippocampus synaptosomes by mianserin enantiomers

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Abstract

In order to test potential links with other stereospecific neurobiological effects of mianserin, the present study explored the stereospecificity for inhibition of depolarization-induced Ca^{2+} uptake by mianserin. Synaptosomes from rat hippocampus were incubated with $^{45}Ca^{2+}$ in either resting or depolarizing (60 mM K^+) choline medium, in the absence or presence (0.6–200 μM) of a mianserin enantiomer. The enantiomers were equipotent (IC_{50} approximating 50 μM) at inhibiting net depolarization-induced Ca^{2+} uptake. This finding, therefore, cannot help to explain the stereoselective enhancement of noradrenaline release by *S*(+)-mianserin; it is also not in keeping with the stereospecificity exhibited by mianserin in acute tests predictive of antidepressant activity, thus suggesting that the calcium-channel blocking activity of mianserin is not linked to its antidepressant effect.

Keywords: Ca^{2+} channel, voltage-dependent; $^{45}Ca^{2+}$ uptake; Hippocampus; Synaptosome; Mianserin, stereoisomer

1. Introduction

A recent study has documented the ability of the atypical antidepressants mianserin, iprindole and fluoxetine to inhibit voltage-dependent Ca^{2+} channels in synaptosomes from the rat hippocampus (Lavoie et al., 1997); fluoxetine and mianserin inhibited depolarization-induced Ca^{2+} uptake into synaptosomes with an IC_{50} of 27 μM and 68 μM, respectively. These IC_{50} values are of the same order of magnitude as the concentrations previously reported for inhibition of the K^+-induced increase in intracellular free Ca^{2+} ($[Ca^{2+}]_i$) in cultured cerebrocortical neurons by mianserin (Shimizu et al., 1992), and for inhibition of the K^+-stimulated increase of $[Ca^{2+}]_i$ in cerebrocortical synaptosomes by fluoxetine (Stauderman et al., 1992). It seems very likely that the inhibition of synaptosomal voltage-dependent Ca^{2+} channels by fluoxetine (Lavoie et al., 1997) can account for the reduction in the K^+-induced rise in synaptosomal $[Ca^{2+}]_i$ (Stauderman et al., 1992). However, for mianserin, a direct correlation of our Ca^{2+} channel results (Lavoie et al., 1997) with the $[Ca^{2+}]_i$ results (Shimizu et al., 1992) is not possible, because of the relatively high expression of L type and low expression of N, P, and Q types of voltage-dependent Ca^{2+} channels in somal membranes as compared with synaptosomal membranes (Elliott et al., 1995).

Mianserin exhibits a strong stereoselectivity for the inhibition of noradrenaline reuptake, the dextrorotatory isomer being more than 100 times more potent than its levorotatory counterpart (Schoemaker et al., 1981). Similarly, the mianserin-induced antagonism of rat muricidal behavior (Schoemaker et al., 1981) and modifications of human electroencephalographic activity (Itil, 1981) appear to reside mostly with the dextrorotatory isomer. The aim of the present study was to ascertain whether the inhibition of synaptosomal voltage-dependent Ca^{2+} channels by mianserin also exhibits stereoselectivity. The K^+-induced release of noradrenaline from slices of rat cerebral cortex is enhanced by racemic mianserin and by the dextrorotatory isomer (Schoemaker et al., 1981; Nickolson et al., 1982); although these authors have attributed this enhancement of noradrenaline release to inhibition of presynaptic α_2-adrenoceptors, the stereoselectivity of the interaction with α receptors (Nickolson et al., 1982) appears much weaker than the stereoselectivity of the enhancement of K^+-induced noradrenaline release. Inhibition of voltage-dependent Ca^{2+} channels could interfere with K^+-induced noradrenaline release; our comparison of the relative potency of mianserin isomers against Ca^{2+} channels thus

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offers a test of whether a preferential inhibition of Ca^{2+} channels by the levorotatory isomer of mianserin could contribute to the marked stereoselectivity of the enhancement of K^+-induced noradrenaline release.

2. Materials and methods

2.1. Chemicals

The *R*(−)-mianserin maleate and *S*(+)-mianserin maleate used in the present study were generously donated by Organon Canada (West Hill, Ontario). The special chemicals used were: HEPES, from Boehringer Mannheim Canada (Montréal, Québec); choline chloride, from A and C American Chemicals (Montréal, Québec); Trizma base and EGTA, from Sigma (St. Louis, MO); sucrose, from Fisher Scientific (Fairlawn, NJ); $^{45}CaCl_2$, specific activity 8.02 Ci/g, from ICN Radiochemicals (Irvine, CA).

2.2. Preparation of synaptosomes

The animals were treated in accordance with the principles and guidelines of the Canadian Council on Animal Care, and the protocol was approved by the University of Montreal animal ethics committee. In each experiment, 5 male Sprague–Dawley rats (200–250 g; Charles River Canada, St-Constant, Québec) were decapitated, and hippocampal tissue was dissected from the isolated brain. Synaptosomes were prepared at 0–4°C as described in Blaustein et al. (1978), the purified synaptosomes being ultimately harvested in the 0.80 M sucrose fraction of a discontinuous sucrose gradient. The purified synaptosomal suspension was diluted 3–4-fold with buffered (pH 7.4) NaCl-based medium (Blaustein et al., 1978), and centrifuged at $15000 \times g$ for 6 min. The pellet obtained from a 3-ml aliquot of the diluted suspension was resuspended in distilled water and used for determination of the protein concentration by the method of Lowry et al. (1951) with bovine serum albumin as the standard. The two pellets originating from the remainder of the diluted synaptosomal suspension were resuspended and pooled in a combined volume of 1 ml of choline-rich solution (mM: choline chloride, 145; KCl, 5; $CaCl_2 \cdot 2H_2O$, 1.2; $MgCl_2 \cdot 6H_2O$, 1.2; Na_2HPO_4, 2.4; HEPES, 10; dextrose, 10), pH 7.4, for use in $^{45}Ca^{2+}$ uptake assays.

2.3. Ca^{2+} uptake assays

Only one putative Ca^{2+} uptake blocking drug was used in any single experiment, and the assignment of a drug to a particular experiment took place according to a predetermined randomized order. Seven 0.1 ml aliquots were withdrawn from the original 1 ml suspension of synaptosomes in choline-rich solution, in order to allocate each aliquot to one of the seven different drug concentrations to be studied. To achieve drug concentrations of 0, 0.6, 2, 6, 20, 60 and 200 μM, a 0.2 ml volume of choline-rich solution containing an appropriate drug concentration was added to the 0.1 ml aliquot of synaptosomal suspension. Then, two 0.1 ml aliquots were immediately withdrawn from each of the seven newly prepared drug-containing suspensions of synaptosomes, and they were preincubated separately for 24 min at 30°C.

A prewarmed (30°C) 0.2 ml volume of the choline-rich medium described above and which also contained $^{45}Ca^{2+}$ (1.5 μCi/μmol Ca^{2+}) was then added to one member of each pair of preincubated synaptosome aliquots. The other member of the pair received a 0.2 ml volume of the following $^{45}Ca^{2+}$-containing high-K^+ choline medium (mM: choline chloride, 62.5; KCl, 87.5; $CaCl_2 \cdot 2H_2O$, 1.2; $MgCl_2 \cdot 6H_2O$, 1.2; Na_2HPO_4, 2.4; HEPES, 10; dextrose, 10). For each pair of aliquots, the drug concentration of the added $^{45}Ca^{2+}$-containing solutions was the same as the one used during the preincubation. The final protein concentration per sample was of the order of 1 mg/ml. Uptake of $^{45}Ca^{2+}$ was allowed to proceed for 10 s at 30°C, and was terminated and quantitated as described previously (Beauchamp et al., 1993).

The amount of $^{45}Ca^{2+}$ taken up during the incubations with choline-rich medium was termed basal uptake whereas that taken up in the course of incubations with depolarizing (60 mM K^+ final) choline medium was referred to as stimulated uptake. For each drug, the results on basal and stimulated uptake were analysed statistically by an analysis of variance adapted for a factorial experimental design (Winer, 1971). Again for each drug, analysis of covariance (Winer, 1971), with basal uptake as regressor and stimulated uptake as regressed variable, was used to derive conclusions applicable to the net K^+-stimulated $^{45}Ca^{2+}$ uptake, which is a specific index of uptake via the voltage-dependent Ca^{2+} channels (Suszkiw et al., 1989). The log of the drug concentration which inhibited 50% of the net $^{45}Ca^{2+}$ uptake [log (IC_{50})] was determined by interpolation of the linear regression between net Ca^{2+} uptake and the log of the three (dextrorotatory isomer) or four (levorotatory isomer) highest drug concentrations for each experiment; the mean log (IC_{50}) values of the compounds were compared by analysis of variance (Winer, 1971). The critical level of probability to accept differences was set at 0.05 for all statistical comparisons.

3. Results

The effects of the drugs under study on $^{45}Ca^{2+}$ uptake in depolarizing choline medium and choline-rich medium are shown in Fig. 1A. In these concentration-dependent studies, inhibition of $^{45}Ca^{2+}$ uptake in the depolarizing choline medium is readily apparent for both isomers of mianserin, reaching close to 60% at 200 μM. A significant

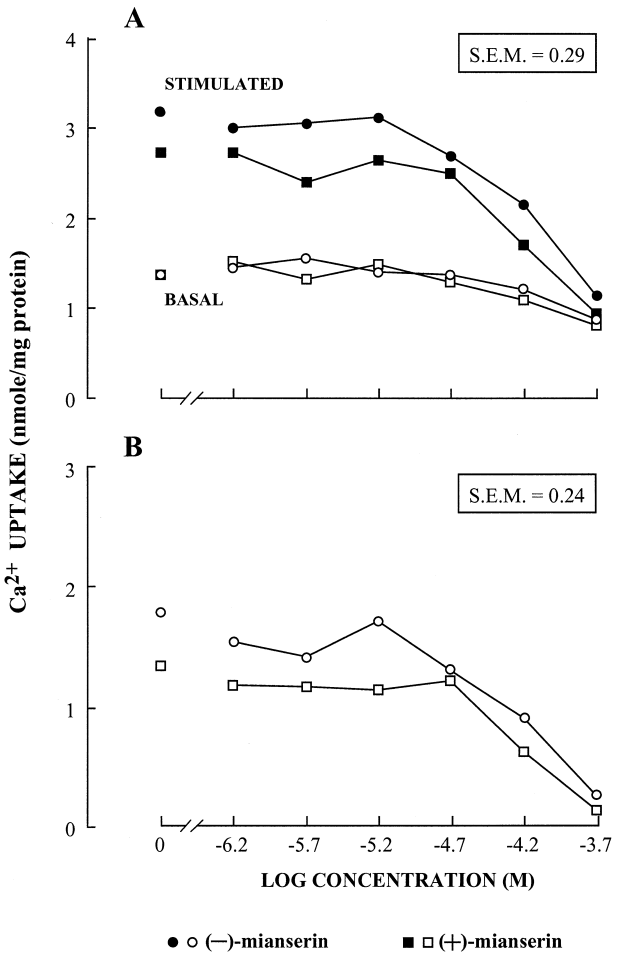

Fig. 1. Effect of mianserin stereoisomers on (A) $^{45}Ca^{2+}$ uptake in choline-rich (basal) and depolarizing choline (stimulated) conditions, and (B) net K^+-induced $^{45}Ca^{2+}$ uptake. The points are the mean of the results from 6 different synaptosomal preparations. In panel B, the Ca^{2+} uptake values were obtained by subtracting the mean choline-rich uptake from adjusted high-K^+ choline uptake values obtained by analysis of covariance. In accordance with analysis of variance (panel A) and analysis of covariance (panel B), an S.E.M. value common to all data points in each panel is presented (see insets).

but quantitatively less important inhibition of $^{45}Ca^{2+}$ uptake is also observed in the choline-rich medium in the upper range of the drug concentrations tested (Fig. 1A).

For each isomer, the slope of the concentration–response curves for $^{45}Ca^{2+}$ uptake in depolarizing choline medium and choline-rich medium differ markedly from one another, such that the observed reductions in $^{45}Ca^{2+}$ uptake in depolarizing choline medium are not matched by parallel reductions in basal $^{45}Ca^{2+}$ uptake. This finding suggests a drug-induced inhibition of the uptake of $^{45}Ca^{2+}$ via the voltage-dependent Ca^{2+} channels activated by K^+-induced depolarization. Drug effects on the net K^+-induced Ca^{2+} uptake were revealed through an analysis of covariance. Both isomers were found to strongly inhibit $^{45}Ca^{2+}$ uptake mediated by Ca^{2+} channels in the hippocampal synaptosomes, as illustrated in Fig. 1B. However, no significant difference could be found, either in the orthogonal comparison between isomers, or by comparison of their respective IC_{50} values (log IC_{50} values for $n = 6$: dextrorotatory isomer, -4.30 ± 0.12; levorotatory isomer, -4.29 ± 0.12). The IC_{50} values for the dextrorotatory and levorotatory isomers were respectively 50 μM and 51 μM.

4. Discussion

The present study demonstrates a clear inhibition of net depolarization-induced $^{45}Ca^{2+}$ uptake by the two optical isomers of mianserin, which is indicative of inhibition of voltage-dependent Ca^{2+} channels by these drugs. As evidenced by their nearly identical IC_{50} values, the stereoisomers of mianserin do not differ in their potency to inhibit the voltage-dependent Ca^{2+} channels of nerve terminals. This is, to our knowledge, the only neurobiological effect, other than binding to rat brain histamine receptors (Nickolson et al., 1982; Pinder and van Delft, 1983), for which no stereoselectivity is observed for the isomers of mianserin.

If inhibition of nerve terminal Ca^{2+} channels had been stereoselective in favor of the levorotatory isomer, this might have helped to explain why the dextrorotatory isomer of mianserin is so much more effective than the levorotatory isomer for enhancing K^+-induced noradrenaline release. However, the similar activity of the mianserin enantiomers against Ca^{2+} channels is not in keeping with the stereoselectivity observed for enhancement of noradrenaline release. The nature of the factor(s), other than inhibition of presynaptic α_2-adrenoceptors, which may play a role in the stereoselective enhancement of K^+-induced noradrenaline release by mianserin therefore remains to be determined.

It has been proposed that an elevation of intracellular free Ca^{2+} could be involved in the etiology of affective disorders, and that antidepressant drugs may operate through normalization of an overactive Ca^{2+} signal (Dubovsky et al., 1992). Reduction of Ca^{2+} inflow through voltage-dependent Ca^{2+} channels represents a potential mechanism for reducing $[Ca^{2+}]_i$, and the present experiments provide a test of the possible link of Ca^{2+} channel inhibition to the therapeutic effect of mianserin. In the inhibition of rat muricidal behavior paradigm for detection of antidepressant activity, the dextrorotatory isomer is active at doses that do not interfere with shuttle box avoidance whereas the levorotatory isomer is not (Schoemaker et al., 1981). This could suggest that the dextrorotatory isomer is a more active antidepressant than its antipode, if not for the fact that the clinically active compound 6-azamianserin (Claghorn et al., 1987) fails to specifically interfere with rat muricidal behavior (Nickolson et al., 1982). Mianserin causes changes in human electroencephalographic activity which closely match those produced by tricyclic antidepressants, an observation which greatly contributed to promote clinical testing of mianserin as an antidepressant (Itil, 1981); the isomers of mianserin

were tested by Itil (1981) along with the racemate, the dextrorotatory isomer producing the electroencephalographic pattern typical of antidepressants at lower doses than the levorotatory isomer. Considering the value that this type of electroencephalographic observation has had for predicting the antidepressant activity of mianserin, the clinical activity of mianserin may well depend more on the dextrorotatory isomer than on its antipode. Inasmuch as the acute tests predictive of clinical antidepressant activity favor the dextrorotatory isomer of mianserin (Itil, 1981; Schoemaker et al., 1981) whereas the mianserin isomers are equipotent for Ca^{2+} channel inhibition, it would appear that the Ca^{2+} channel blocking activity of mianserin is not linked to its therapeutic effect.

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