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Short communication

Inhibition of SK3 channels in the TE671 human medulloblastoma cell line by desipramine and imipramine

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Abstract

The TE671 human medulloblastoma cell line endogenously expresses SK3 channels. Using patch clamp, we tested the effects on this current of desipramine and imipramine. In both cases, we observed a complete, reversible and concentration-dependent block. The interaction of desipramine with the selective SK3 blocker, apamin, was studied in more detail. Co-application of desipramine and apamin at concentrations close to their IC_{50} produced an additive effect that was significantly higher than that of each compound alone. This effect was also observed at IC_{25} concentrations. Collectively, these data provide evidence against a common site of action for desipramine and apamin.

Keywords: TE671 cell line; SK3 channel; Antidepressant, tricyclic; Desipramine; Imipramine

1. Introduction

Tricyclic antidepressant drugs are extensively used for the treatment of depression, panic disorder, bulimia and obsessive compulsive disorder (Daly and Wilens, 1998). Their action is suggested to be mediated through inhibition of reuptake of 5-hydroxytryptamine and noradrenaline at the synaptic cleft (Hollister, 1995). However, they have a number of other effects such as blockade of muscarinic (Snyder and Yamamura, 1977), nicotinic (Rana et al., 1993) and histamine receptors (Stahl, 1998). Finally, tricyclic antidepressants are also effective in blocking various ion channels such as the rapidly inactivating K^+ channels of rat hippocampal neurones (Kuo, 1998), L-type Ca^{2+} channels in mouse dorsal root ganglia (Choi et al., 1992), Na^+ channels of rat sensory neurons (Song et al., 2000) and rat recombinant SK2 channels (Dreixler et al., 2000). Furthermore, tricyclic antidepressant drugs such as desipramine and imipramine, have been reported to block the human recombinant SK3 subtype of Ca^{2+}-activated K^+ channels (Terstappen et al., 2001) and binding data suggested a direct and competitive interaction of these compounds with the apamin binding site. The TE671 human medulloblastoma cell line has been recently demonstrated to endogenously and selectively express SK3 channels (Carignani et al., 2002). In this study, with the use of patch-clamp electrophysiology, we investigated the effect of two tricyclic antidepressants (desipramine and imipramine) on the SK3 current of TE671 cells with particular attention in the interaction of desipramine with the apamin site.

2. Materials and methods

2.1. Cell culture

Human TE671 cells were obtained from American Type Culture Collection (Manassas, VA, USA). The cells were cultured as previously described (Carignani et al., 2002). Briefly, cells were grown in DMEM supplemented with 10% heat-inactivated fetal calf serum (both from Life Technologies, Rockville, MD, USA) and plated at a density of 40,000 cells/ml onto glass coverslips inside 35-mm Petri dishes.

2.2. Electrophysiology

Electrophysiological recordings were made from days 2 to 4 after plating. Cells were voltage clamped using an Axopatch 200 amplifier (Axon Instruments, Foster City,

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CA, USA). Patch pipettes were pulled to a tip diameter of 2–3 μm which gave a resistance of around 5–8 MΩ when filled with the following intracellular solution (in mM): KCl, 140, EGTA, 10, and HEPES, 10, at pH 7.3. $CaCl_2$ and $MgCl_2$ were added in such concentrations to obtain a free cytosolic Ca^{2+} concentration of 437 nM and 1.1 mM free Mg^{2+}. The estimation of the concentrations of free Ca^{2+} and Mg^{2+} was performed by means of winMAXC (Stanford University, Pacific Grove, CA, USA). For electrophysiological recordings, the culture medium was changed to the following extracellular solution (K-140) containing (in mM): KCl, 140, $MgCl_2$, 1, $CaCl_2$, 2, HEPES, 10, and glucose, 7.5, at pH 7.3. A similar solution containing 140 mM NaCl in the place of KCl (Na-140) was used for estimating the amount of leakage as already described (Carignani et al., 2002). Data were digitized (10 kHz), filtered (5 kHz) and stored online using the pCLAMP 8.1 software and Digidata 1320A interface (Axon Instruments).

2.3. Data analysis

Data analysis was carried out using pCLAMP 8.1 (Axon Instruments) and GraphPAD Prism (GraphPAD software, San Diego, CA, USA). Experimental data are reported as means ± S.E.M. P values [with significance levels at $p < 0.001$ (**)] were calculated using the one-way ANOVA followed by the Dunnett's Multiple Comparison Test. Data in the antagonist concentration–response relationships represent values normalized with respect to the currents in the absence of antagonist and were fitted to the following logistic equation:

$$Y = \mathrm{MIN} + (\mathrm{MAX} - \mathrm{MIN})/(X^{n_H} + \mathrm{IC}_{50}^{n_H})$$

where MAX and MIN are the maximal and minimal current blockade (in percent values), IC_{50} is the concentration of compound producing 50% of inhibition and n_H is the curve slope factor.

Fitting parameters are expressed followed by 95% confidence limits (95% C.L.).

2.4. Drugs and chemicals

Desipramine and imipramine were dissolved, respectively, at 0.01 and 0.1 M in distilled water. Apamin was dissolved in a 0.1 mM stock solution in water containing 0.1% bovine serum albumin (BSA) and diluted into K-140 containing 0.01% BSA. All drugs and salts were obtained from Sigma (St. Louis, MO, USA).

3. Results

SK3 currents were completely blocked in a reversible and concentration-dependent manner by apamin (0.01–1000 nM), desipramine and imipramine (0.001–1000 μM) as shown in Fig. 1. The IC_{50}s obtained from the concentration–response curves were 4.5 nM (95% C.L. 1.7–11.8 nM) for apamin, 30.06 μM (95% C.L. 24.52–36.84 μM) for desipramine and 31.89 μM (95% C.L. 22.84–44.51 μM) for imipramine. With the aim of studying the possible existence of a common site of action for apamin and the tricyclic desipramine, we studied the effect of co-application of the drugs. Currents were evoked in the presence of desipramine

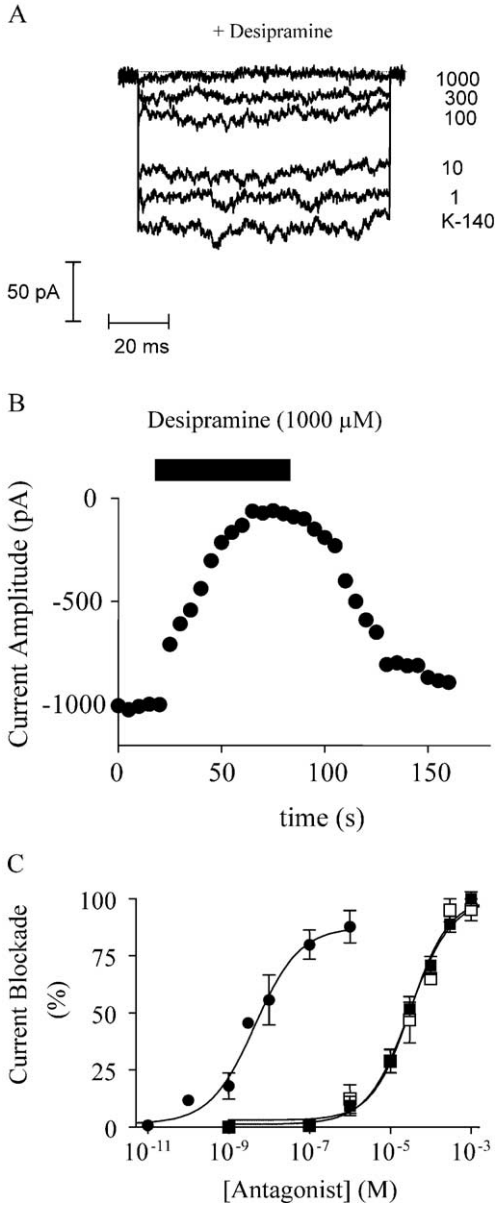

Fig. 1. Effect of desipramine, imipramine and apamin on SK3 currents. (A) Membrane current recording shows the inhibitory effect of desipramine (at the μM concentrations indicated aside) on a representative cell. The dashed line indicates the level of zero current. (B) Time course of the effect of desipramine 1000 μM on the SK3 current in a representative cell. The bar represents the time of drug application. (C) Concentration–response curves of desipramine (■), imipramine (□) and apamin (●). Data points are the mean of responses from to 4–10 cells for each concentration of desipramine, 4–9 for each concentration of imipramine and 4–12 for each concentration of apamin.

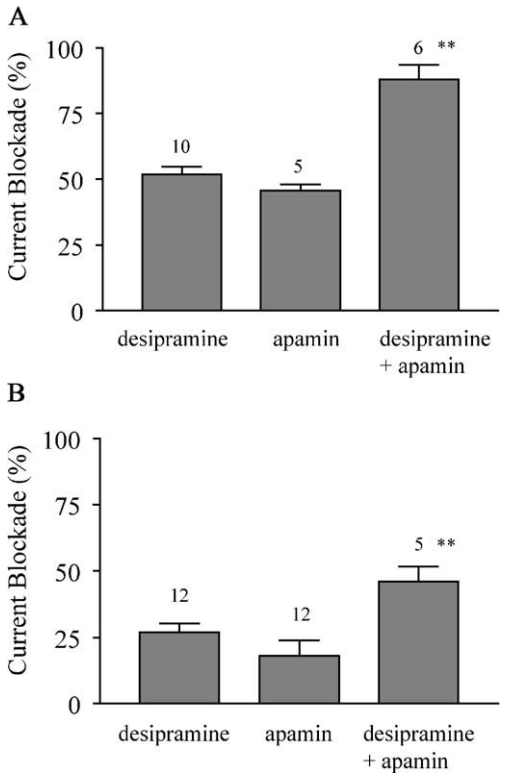

Fig. 2. Effect of co-application of desipramine and apamin on SK3 current. The number of experiments is indicated above each column, together with the S.E.M. bars and the level of significance ($p<0.001$ for desipramine+apamin vs. desipramine or apamin). (A) Concentrations close to the IC_{50} of desipramine (30 μM) and apamin (0.003 μM). (B) Concentrations close to the IC_{25} of desipramine (10 μM) and apamin (0.001 μM).

(30 μM) and apamin (0.003 μM), both at concentrations close to their respective IC_{50}s. The combination produced an inhibition of the SK3 currents of 87.7 ± 5.7% ($n=6$). As shown in Fig. 2A, this amount of inhibition was significantly higher ($p<0.001$) than the value of both compounds alone (51.6 ± 3.1%, $n=10$ for desipramine and 45.6 ± 2.3%, $n=5$ for apamin) and close to the sum of the two effects. The same kind of statistically significant ($p<0.001$) effect (Fig. 2B) was reported when applying concentrations of desipramine (10 μM) and apamin (0.001 μM) close to the IC_{25}. The amount of inhibition in this case was 46.0 ± 5.6% ($n=5$) in the presence of both compounds, whereas the percentage of inhibition was 28.8 ± 3.3% ($n=12$) in the presence of desipramine and 18.0 ± 5.7% ($n=12$) in the presence of apamin alone.

4. Discussion

A number of publications reported the variety of effects of tricyclic antidepressants other than the principal action of inhibition of neurotransmitter uptake. In particular, among the effects on many ionic channels (Choi et al., 1992; Kuo, 1998; Song et al., 2000), the block of rat brain recombinant SK2 (Dreixler et al., 2000) and SK3 (Grunnet et al., 2001) channels is of particular interest because of the distribution in central neurons of these channels. In addition, tricyclic antidepressants have been reported to inhibit also human recombinant SK3 channels (Terstappen et al., 2001), using both a fluorescence assay and binding experiments. TE671 cells have been previously demonstrated to express selectively the subtype 3 of these channels, SK3 (Carignani et al., 2002). The data presented in this study show through electrophysiological measurements, that the tricyclic antidepressants desipramine and imipramine block the native SK3 current of TE671 cells. The block produced by desipramine and imipramine was reversible and concentration-dependent and the potency of the two drugs was substantially the same, the IC_{50}s being 30.06 μM for desipramine and 31.89 μM for imipramine. These concentrations are about 30-fold the plasma concentration of around 1 μM reached by tricyclic antidepressants during daily therapy (Baldessarini, 1985). On the other hand, these drugs have been reported to accumulate up to 20-fold in the human brain after chronic treatment (Kamatchi and Ticku, 1991), thus reaching quite easily a concentration close to the IC_{50} identified in this work. The action of one of these drugs on the SK3 channel was studied in more detail considering the interaction of desipramine with the apamin site. A binding study on CHO-K1 cells transfected with the human SK3 channel (Terstappen et al., 2001) had already reported the displacement of [^{125}I]-apamin binding by desipramine, thus suggesting an interaction between these two compounds with SK3 channels. A simple way to explain the binding data is to consider a common binding site for apamin and desipramine. However, this hypothesis is not fully corroborated by our observations, although they were obtained in two different cell lines. In fact, the evidence that adding apamin together with desipramine produced a final effect on SK3 current that is the sum of the single inhibition of the compounds tested alone argues against the hypothesis of a common functional interaction site. The additive effect was robust since it was produced by two dose levels of apamin and desipramine (IC_{50} and IC_{25}). A preliminary consideration on the mechanism of action of inhibition by desipramine can be done considering that an indirect action of desipramine on the SK3 channel through a possible inhibition of Ca^{2+} influx (reported for desipramine by Lavoie et al., 1990 and for imipramine by Shimizu et al., 1994) seems not to be the case. In fact, in our experimental protocol, the SK3 channels are kept constantly activated by the intracellular Ca^{2+} concentration, buffered to a sufficiently high level (437 nM) by the pipette solution and are thus independent of Ca^{2+} influx.

In summary, it can be concluded that the SK3 channels of TE671 cells can be completely, reversibly and dose-dependently inhibited by two tricyclic antidepressants such as desipramine and imipramine. Furthermore, desipramine seems to act on SK3 channels to a site able to modify the SK3 channel functionality and, potentially, the apamin binding. Thus, it could have an allosteric interaction with

the SK3 channel–receptor complex. This is not surprising considering that similar conclusions have already been drawn for related potassium channels such as BK of mouse (Zhang et al., 2001). Finally, this block occurs at micromolar concentrations that are close to the therapeutic plasma concentration of tricyclic antidepressants in the brain. These observations acquire a particular relevance when the SK3 distribution is considered. In fact, SK3 channels have been reported (Rimini et al., 2000, Stocker and Pedarzani, 2000, Tacconi et al., 2001) to be particularly highly expressed in such cerebral areas as dorsal raphe, locus coeruleus and substantia nigra pars compacta which are of particular interest for neuropsychiatric disorders. Indeed, these areas play a central role in serotonergic, noradrenergic and dopaminergic transmissions and are considered to fail in their function in depression disorders. Therefore, the selective presence of SK3 channels in dorsal raphe, locus coeruleus and substantia nigra pars compacta could suggest an interaction with tricyclic antidepressant drugs which, by blocking the channel, could facilitate the neuronal firing and thus contribute to their clinical effects.

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