Pyridoindole Stobadine is a Nonselective Inhibitor of Voltage-Operated Ion Channels in Rat Sensory Neurons

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Abstract. Pyridoindole stobadine, a compound with known neuroprotective, antioxidant, oxygen-free radical scavenging and antiarrhythmic properties, was analysed as to its effect on inward sodium and calcium currents (I_{Na} and I_{Ca}), respectively, and on fast inactivating and slow non-inactivating components of potassium outward current (I_{Kf} and I_{Ks}), respectively, in the neuronal membrane. A voltage-clamp technique was used in internally dialyzed single neurons isolated from young rat sensory ganglia.

Stobadine inhibited all currents studied in a concentration dependent manner (0.1–3 mmol/l). Apparent affinity constants pD'_2, – (i.e. negative logarithm of IC_{50}) were 3.147 ± 0.030, 3.112 ± 0.084, 3.089 ± 0.062 and 2.918 ± 0.059 for I_{Na}, I_{Ks}, I_{Kf}, and I_{Ca}, respectively. Full current inhibition occurred only in I_{Na} at concentrations of > 1 and ≤ 3 mmol/l. Saturation of the inhibition of both components of I_K at approximately 40% of the control level occurred at concentrations > 1 mmol/l.

Stobadine was found to be a nonspecific inhibitor of the currents studied. This property as well as the range of pD'_2, values are comparable to those observed in the local anesthetics procaine and trimecaine. The inhibition of voltage-operated ion channels in membranes of excitable cells by stobadine might be responsible for its capability to inhibit synaptic transmission and axonal conduction. It might participate also in the antidysrrhythmic effect of stobadine.

Key words: Sensory neuron membrane — Na^+ current — Ca^{2+} current — K^+ currents — Stobadine — Free radical scavenger — Antiarrhythmic agent — Cell dialysis — Voltage-clamp

Introduction

Stobadine, a compound with pyridoindole structure (Beneš and Štolc 1989), was found to protect synaptic transmission in the rat superior cervical ganglion and hippocampus against hypoxia-reoxygenation induced injury (Štolc and Selecká 1993,

1994; Štolc 1994). This effect, observed at the concentration of 10 μmol/l, was linked to antioxidant and free radical scavenging properties of the compound (Mišík et al. 1991; Steenken et al. 1992; Štefek et al. 1992; Kagan et al. 1993). At concentrations exceeding the above given one, stobadine inhibited synaptic transmission in both neuronal structures in a concentration dependent manner. Accordingly, the conduction of action potential in the rat preganglionic sympathetic nerve was diminished in the presence of stobadine at the concentration of 1 mmol/l (Štolc and Selecká 1993; Štolc 1994). Moreover, stobadine revealed a powerful antiarrhythmic property (Bauer et al. 1982; Štolc et al. 1982; Gibala et al. 1985; Kittová et al. 1985).

The aim of the present study was to analyse on the neuronal membrane whether stobadine was able to affect the ion currents passing through sodium, calcium and potassium voltage-operated channels.

Materials and Methods

Materials

Stobadine [(-)-cis-2,8-dimethyl-2,3,4,4a,5,9b-hexahydro-1H-pyrido(4,3b)indole dihydrochloride] was supplied by Dr. L. Beneš (Institute of Experimental Pharmacology, Slovak Academy of Sciences, Bratislava). Trypsin (Trypsin® SPOFA), crude collagenase (ÚSOL, Bohumile, Czech Republic) and other chemicals (all of them of analytical purity) were obtained from commercial sources.

Methods

A voltage-clamp technique in single neurons isolated from dorsal root ganglia of young rats (5–10 days old) was used. The cells were fixed in a perfusing system allowing to control the composition of extracellular and intracellular media. The modified version used in this study was derived from the technique developed by Kostyuk et al. (1981) and was described in detail elsewhere (Štolc et al. 1988, Štolc 1993). Briefly, neurons were dissected free from the ganglia following enzymatic treatment (trypsin 8 mg/ml with collagenase 1000 U/ml for 15 min at 32 °C). Single cells were wedged into the conical pore in the septum separating "external" and "internal" compartments of the perfusing system. Following rupture of that part of the cell membrane which was facing the "internal" compartment, the composition of solutions on both sides of the effective part of the membrane could be controlled easily. The solutions on both sides of the membrane were continuously flowing at a rate of 0.5–0.7 ml/min. The normal extracellular medium consisted of NaCl 140, $CaCl_2$ 2, $MgCl_2$ 2, Tris-Cl 5 (in mmol/l), while the intracellular one of KF 70, Tris-F 50, glucose 20, EDTA 1. The sodium current (I_{Na}) was isolated from the total current by equimolar replacement of intracellular K^+ by Tris. Correspondingly, the potassium current (I_K) was obtained following replacement of extracellular Na^+ by Tris. When observing the calcium current (I_{Ca}), the extra- and intracellular media consisted of $CaCl_2$ 14, $MgCl_2$ 2, Tris-Cl 133 and Tris-F 140, ATP 2, cAMP 0.05, respectively. Time- and voltage-characteristics of these currents were identical with those described and documented elsewhere (Štolc 1993). The pH of all solutions was adjusted by appropriate acid (HCl or HF) to 7.3. The experiments were carried out at 18 °C.

The inward I_{Na} and I_{Ca} were evoked by depolarizing pulses lasting 30 ms (Fig. 1*B*). The maximal available amplitude was followed up in these two currents at the frequency of 0.1 Hz before and during application of the drug. The holding potentials used were −120 mV and −100 mV in analysis of I_{Na} and I_{Ca}, respectively. The outward I_K was evoked by arbitrarily selected depolarization to 0 mV (300 ms pulses at 0.1 Hz). The holding potential was −120 mV. The peak current amplitude appearing in the early phase of the outward current was considered to be its fast inactivating component (I_{Kf}). The steady-state current occurring at the end of the depolarization pulse was considered to be its slow non-inactivating component (I_{Ks}) (Štolc 1993). Compensation of the leakage current was adjusted manually at hyperpolarizing pulse. Effect of series resistance was minimized by reducing the size of effective membrane patch resulting in an increase at a ratio R_m/R_s by a procedure described elsewhere (Štolc et al. 1988; Štolc 1993). The viability of the cells over the experimental period was checked in series of control cells from each batch. Under the conditions used, no current recorded was decaying by more than 10–15% for a period longer than 20 min. Rather, an increase in current amplitudes was frequently observed. Stobadine was applied into the extracellular medium for 20 min. This time interval was long enough to reach a steady-state effect that was used to evaluate the concentration-effect relationship. For each concentration tested and each current analysed, 6–13 separate cells were used. Apparent dissociation constants pD_2' (i.e. negative logarithms of middle inhibitory concentrations IC_{50}) with estimates of their errors were calculated from the linear part of the relationships according to Grimm (1973). They were considered to be measures of the apparent drug affinity to particular ion channels.

Results

The effect of stobadine on I_{Na}, I_{Ca}, I_{Kf}, and I_{Ks} was studied in the concentration range from 0.1 to 3 mmol/l. Typical records of the currents analysed are shown in Fig. 1*B*. The I_{Ca} observed was conducted mainly *via* L-type channels, while the I_{Kf} and I_{Ks} seem to correspond to the A-current and delayed rectifier, respectively (Štolc 1993). The effect of stobadine on all the currents investigated was developing gradually and became stabilized whithin 5–15 min depending on the concentration tested. This comparatively slow drug effect development might be partially ascribed to the slow increase of drug concentration in the extracellular compartment following switching from the normal to the drug-containing perfusion medium. The dependence of the inhibitory effect of stobadine on the compound concentration for each particular current measured after 20 min of its application is shown in Fig. 1*A*. The lowest concentration evoking statistically significant inhibition of I_{Na} was between 0.3 and 1 mmol/l, which was approximately by one half of an order higher than that for the other currents. Full inhibition of I_{Na} was observed at 3 mmol/l. Although the other currents were significantly diminished already at 0.3 mmol/l, they were not fully inhibited even at the highest concentration tested. The slope of the linear regression line fitting the middle part of the concentration-effect relationship for I_{Na} (Table 1) was significantly steeper ($p < 0.001$) than the slopes of the relationships of the other currents studied. The slope of the relationship

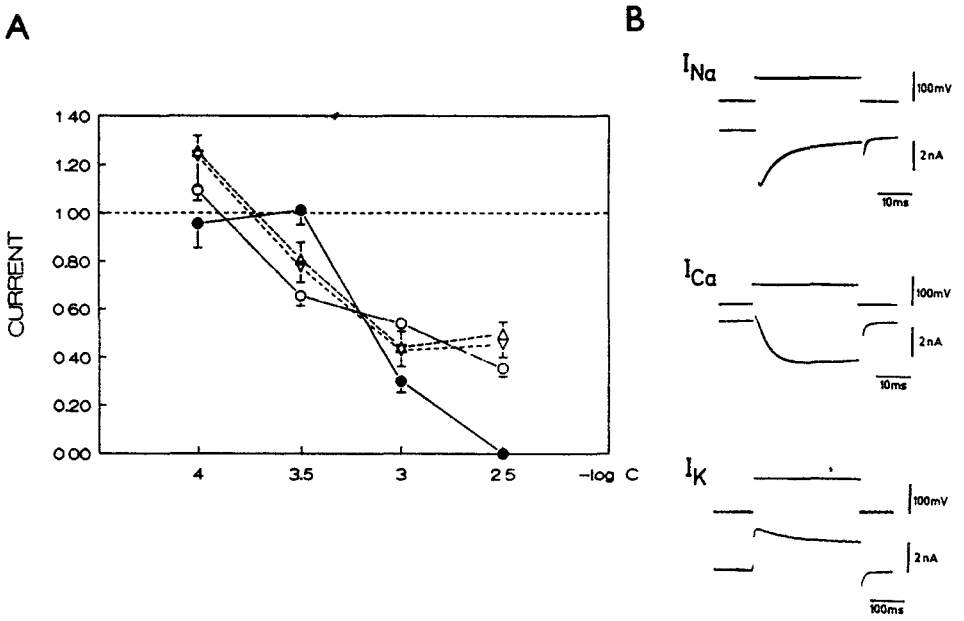

Figure 1. *A* Changes of two inward currents (I_{Na}, ● and I_{Ca}, ○) and two components of the outward current (I_{Kf}, Δ and I_K, ▽) following 20 min application of stobadine at different concentrations. Abscissa: negative logarithms of concentration expressed in mol/l; ordinate: the currents normalized to the values measured before the application. Each point was calculated from measurements on 6–13 cells. Means ± S.E.M. are shown. *B* Representative records of currents evaluated: Inward sodium current, I_{Na}; inward calcium current, I_{Ca}; and outward potassium current with the fast inactivating component (peak value), I_{Kf}, and the slow noninactivating component (steady-state value), I_K. Upper traces: membrane potential; lower traces: transmembrane current. Depolarizing pulses evoking the current with the maximal amplitude were used in both inward currents, while an arbitrary depolarization to 0 mV was used in the outward current. Size of the effective membrane patch was approximately 10 times smaller in I_{Na} and I_K than in I_{Ca} (Štolc et al. 1988)

for I_{Ca} was significantly less steep than the other ones ($p < 0.001$). The break in the linear part of the concentration-effect relationship for both components of I_K occurring at 1 mmol/l might indicate saturation of the inhibitory effect at higher concentrations.

The slight stimulatory effect of stobadine on I_{Ca} and both components of I_K occurring at the concentration of 0.1 μmol/l is only apparent. A similar increase in current amplitude was observed also in most control cells at this time interval.

The apparent affinities of stobadine to the channels involved in the currents studied are shown in Table 1. As the differences between the pD'_2 values did

Table 1. Regression coefficients b characterizing the slope of the linear part of the dependence of stobadine effect on the negative logarithm of its concentration (mol/l) with its error S_b. Apparent affinity constants pD'_2, of stobadine ($-\log IC_{50}$) with estimates of error to voltage-operated channels in somatic membranes of young rat sensory neurons conducting the indicated ion currents.

	I_{Na}	I_{Ca}	I_{Kf}	I_{Ks}
b	−1.364*	−0.471*	−0.806	−0.798
S_b	±0.144	±0.043	±0.091	±0.125
pD'_2	3.147	2.918	3.089	3.112
	±0.030	±0.059	±0.062	±0.084

* – difference to all other corresponding values significant at $p < 0.001$.

not exceed one third of a unit, stobadine should be characterized as a nonspecific inhibitor of the currents studied in the neuronal somatic membrane.

Discussion

The capability of stobadine to diminish the injury of nervous tissue induced by hypoxia and reoxygenation was demonstrated on several models *in vivo* and *in vitro* (Štolc and Horáková 1988; Horáková et al. 1990, 1991; Lukáčová et al. 1993; Štolc and Selecká 1993, 1994; Štolc 1994). Moreover, this compound was observed to inhibit both synaptic transmission in the rat superior cervical ganglion and hippocampus and action potential conduction in the rat preganglionic nerve. The concentrations inducing threshold inhibition, depending on the type of the tissue, were however 3 to 100 times higher than the concentration providing nervous tissue protection (10 μmol/l) (Štolc and Selecká 1993, 1994; Štolc 1994). The difference between the protecting and the inhibitory concentrations may be suggestive of different mechanisms involved in the two actions. The former seems to be related to the antioxidative and free oxygen radical scavenging properties of stobadine (Mišík et al. 1991; Steenken et al. 1992; Štefek et al. 1992; Kagan et al. 1993). The mechanism of the latter had not been analysed previously.

The present study demonstrated concentration-dependent inhibition of voltage-operated channels conducting I_{Na} by stobadine. This effect could substantiate the above mentioned inhibition of synaptic transmission and/or axonal conduction. A similar effect on I_{Na} was observed in identical neurons with the local anesthetics procaine and trimecaine (Štolc 1988). Besides their effect on I_{Na}, these compounds inhibited also both components of I_K with the corresponding pD'_2, values not differing more than approximately by one unit (Štolc 1988). A comparable non-specific effect of stobadine was observed in the present study. The overall range of the

effective inhibiting concentrations of stobadine and procaine is also very similar (Štolc 1988).

The differences between the slopes of the middle part of the concentration-effect regression lines for particular currents obtained with stobadine seem to be compatible with the view that the currents studied are conducted *via* different channel entities with different drug-receptor interactions. Similar results were obtained also with the local anesthetics procaine, trimecaine and carbisocaine (Štolc 1988). The saturation of the effect of stobadine on both components of I_K at concentration > 1 mmol/l resembles the irregularity in the concentration-effect relationship observed in the effect of the carbanilic drugs carbisocaine and BK 129 on the same current (Štolc 1988). This might indicate a complex interaction between the drugs at low and high concentrations and particular channels, which however would require a more detailed analysis.

The apparent partition coefficient P' of stobadine in the system octanol/phosphate buffer at pH 7.0 is 7.2 (Bezáková et al. 1993). This value is within the range for typical local anesthetics, such as procaine ($P' = 0.6$), lidocaine ($P' = 2.9$) or bupivacaine ($P' = 27.5$) (Covino and Vassallo 1976). Stobadine, thanks to its suitable lipo-hydrophilicity, might incorporate into the lipid part of the cell membrane and/or into channel proteins in a similar way as typical local anesthetics do. This may modify the function of the ion channels involved, resulting in decrease of neuronal excitability.

One of the other known pharmacological properties of stobadine observed so far (for review see Beneš and Štolc 1989) is its antiarrhythmic effect (Bauer et al. 1982; Štolc et al. 1982; Gibala et al. 1985; Kittová et al. 1985), which however has not been submitted to detailed electrophysiological analysis. Nevertheless, the mechanism of action of many antiarrhythmics is known to involve the decrease in the excitability of myocardial cells due to inhibition of various ion channels (Vaughan Williams 1989; Task Force 1991).

It is suggested that the capability of stobadine to inhibit neuro-neuronal synaptic transmission and action potential conduction in axons as well as its antidysrrhythmic effect on the myocardium might have a common denominator, i.e. inhibition of voltage operated ion channels in excitable membranes of the cells involved. The dual action of stobadine, namely the protective antihypoxic as well as its local anesthetic-like effect related to inhibition of some voltage-operated ion channels should be taken into consideration when speculating on the use of this compound as a prospective therapeutic agent.

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