Blocking of Neuronal Nicotinic Acetylcholine Receptors with d-Sparteine Derivatives

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The effects of d-sparteine (d-SP) and its two derivatives, N-methylsparteine (IEM-1820) and N-phenylsparteine (IEM-1821), on nicotinic acetylcholine receptors (nAChR) of the rat superior cervical ganglion neurons were studied. Membrane currents evoked by iontophoretically applied acetylcholine were recorded using the patch-clamp recording technique in the whole-cell configuration. All three compounds were found to block nAChR competitively, the blocking activity being increased with an increase in the size of the blocking molecule. The EC50 values for d-SP, IEM-1820, and IEM-1821 were equal to 2.06 ± 0.38 μM (n = 3), 1.64 ± 0.41 μM (n = 4), and 0.65 ± 0.17 μM (n = 3), respectively. It was assumed that the increase in efficiency of blocking is related to the decrease in the rate of dissociation of the blocker and receptor molecules.

INTRODUCTION

The effects of d-sparteine (d-SP) on nicotinic acetylcholine receptors (nAChR) of the rat superior cervical ganglion neurons were studied earlier [1-3]. The d-SP molecule consists of four heterocyclic rings connected to each other with rigid bonds. At neutral pH (7.1-7.4), one of the two tertiary nitrogen atoms is protonated, and the whole molecule is positively charged [4]. Studies of d-SP’s influence on the effects of preliminary activation of nAChR, how d-SP affects the nAChR channel open time, and the acetylcholine (ACh) dose–response relationship suggest that the main mechanism underlying the effect of d-SP on nAChR is a competitive nAChR blockade. It should be noted that d-SP still possesses weak open-channel blocking activity, as judged by small d-SP-induced shortening of excitatory postsynaptic current (EPSC) decay phase and voltage dependence of the d-SP-induced blockade of nAChR [2]. One of the factors that can be responsible for the lack of significant open-channel blocking action of d-SP, in contrast to many other ganglionic blocking drugs, is the large size of the d-SP molecule, which exceeds the size of the nAChR open channel [5, 6]. Latest studies revealed that hyperpolarization of neuronal membrane decreases the inhibitory constant characterizing the effect of d-SP on nAChR, which may be due to the increased affinity of nAChR for the blocker, and may explain the voltage dependence of the d-SP blocking effect. One possibility is that the d-SP molecule, when interacting with nAChR, is partially submerged in the neuronal membrane, which makes its interaction with the recognition center of nAChR voltage sensitive [3].

If this interpretation is correct, one can expect that further increase in the size of d-SP-like molecule, even if it modifies the inhibitory effect of d-SP on nAChR, should not be followed by an increase in its channel-blocking activity.

Only one of the d-SP derivatives, an alkaloid lupanine, has been tested for its blocking effect on ganglionic nAChR [7]. The lupanine molecule is slightly larger than that of d-SP, and its inhibitory constant is somewhat higher than in d-SP (500 and 400 nM, respectively).

In this work, the effects of two synthetic d-SP derivatives, N-methylsparteine (IEM-1820) and N-phenylsparteine (IEM-1821), on ganglionic nAChR have been investigated.
METHODS

Experiments were performed on the neurons of rat isolated superior cervical ganglion. Membrane currents evoked by ACh applied iontophoretically through a micropipette (ACh currents) were recorded using the whole-cell patch-clamp technique [8]. The experimental procedure was described in detail earlier [1, 3]. The extracellular solution had the following composition (mM): NaCl, 133; MgCl2, 1.2; CaCl2, 2.5; tris(oxymethylaminomethane), 10; glucose, 11; pH 7.4. The solution filling a patch pipette had the following composition (mM): KCl, 140; EGTA-KOH, 11; HEPES-KOH, 10; pH 7.2. The experiments were performed at room temperature (20-22°C). The compounds IEM-1820 and IEM-1821 were synthesized by Dr. V. Gmiro in the Institute of Experimental Medicine (Saint Petersburg, Russia).

RESULTS AND DISCUSSION

The compounds IEM-1820 and IEM-1821 are quaternary derivatives of d-SP with molecules larger than that of d-SP: IEM-1820 has an additional methyl group, while IEM-1821 has an additional six-carbon ring connected to a nitrogen atom.

Both compounds reversibly suppressed ACh currents (Fig. 1). Blocking activity increased with an increase in the size of a blocking molecule. The EC50 values (concentration of a blocker corresponding to a 50% blockade) for d-SP, IEM-1820, and IEM-1821 were 2.06 ± 0.38 μM (n = 3), 1.64 ± 0.41 μM (n = 4), and 0.65 ± 0.17 μM (n = 3), respectively. These EC50 values were obtained at a holding potential of -50 mV. The blockade developed during about 3 min, determined mainly by the solution exchange time. The washing-out procedure took three to five times longer than the blockade development.

To clarify the mechanism underlying the blocking action of the d-SP derivatives, the conventional methods allowing us to distinguish between the competitive and the open channel-blocking mechanisms were used. These were the method of paired ACh applications, the investigation of the dependence of the block on membrane potential level, and the investigation of how the blocker modifies the dose-response relationship [9].

For technical reasons, the minimum interval between the two ACh applications was not shorter than 0.7 sec. Only the results obtained when both ACh currents were equal to each other in the absence of a blocker (i.e., when no desensitization was observed) were taken into consideration. No decrease in the amplitude of the second ACh current, if compared with that of the first ACh current, was observed at the blocker concentration producing more than a 50% decrease in the ACh current amplitude (Fig. 2). This result suggests that either there is no open channel block, or the block lasts a very short time, with an unblocking time constant less than 0.3 sec (i.e., IEM-1820 and IEM-1821 are not "slow" open channel blockers). In this relation, the above two blockers are similar to d-SP, which likewise did not yield any use-dependent block at the 0.7 sec or longer interpulse intervals.