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Effects of verapamil on synaptic transmission in mammalian sympathetic ganglia[1,2]

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ABSTRACT Effects of verapamil (Ver) on the synaptic transmission of isolated superior cervical ganglia (SCG) were investigated by means of intracellular recording techniques. In the neurons of SCG of rabbits and guinea pigs, Ver (100-400 μmol / L) depressed both the synaptic responses (f-EPSPs and / or evoked action potentials) elicited by orthodromic stimulation and the acetylcholine (ACh) potentials induced by pressure ejection of ACh. 4-Aminopyridine (4-AP, 100 μmol / L) and $BaCl_2$ (5 mmol / L) facilitated the f-EPSPs inhibited by Ver, but had no notable effect on the ACh potentials depressed by Ver. Ver suppressed the ACh potentials enhanced by pyridostigmine (1 μmol / L). The potentiating effect of soman (1 μmol / L) on the ACh potentials was prevented by Ver. It is suggested that Ver can block the synaptic transmission of mammalian SCG by both the pre- and postganglionic mechanisms.

KEY WORDS verapamil; sympathetic ganglia; aminopyridines; barium; pyridostigmine bromide; soman

The calcium channel blocker verapamil (Ver) exerts a depressant effect on neuromuscular transmission in man and in animal[1,2]. In the sympathetic ganglion, however, the question whether or not Ver exerts a similar effect on synaptic transmission remains to be studied. Ver depresses the 3-aminopyridine-induced synchronous, repetitive postganglionic discharges in frog sympathetic ganglia and the results suggest that Ver can block the ganglionic transmission[3]. The purpose of present study was to investigate the effects of Ver on mammalian sympathetic ganglia and to identify the sites and the mechanisms of action.

MATERIALS AND METHODS

Rabbits, ♂, 1.75± SD 0.25 kg, and guinea pigs, ♂ 275 ± 25 g were used. The superior cervical ganglia (SCG) with its cervical sympathetic trunk were excised and superfused with Krebs solution of the following composition (mmol / L): NaCl 117, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $NaHCO_3$ 25, NaH_2PO_4 1.2, and glucose 11. The solution was gassed with 95% O_2 + 5% CO_2 at 34-35℃.

Intracellular recordings were obtained from neurons of the SCG by means of fiber-containing glass microelectrodes filled with KCl (3 mol / L) having an impedance of 30-50 MΩ. The cervical sympathetic nerve trunk was drawn into a suction electrode for orthodromic stimulation. The fast excitatory postsynaptic potentials (f-EPSPs) were evoked by stimulating the nerve trunk at a frequency of 0.1 Hz. Signals amplified with a WPI 707A preamplifier were displayed on a Tektronix oscilloscope and recorded on a Gould pen recorder. The direct simulation to the ganglionic neuron was applied through the recording electrode. Acetylcholine (ACh) was applied to the ganglion cells by pressure ejection (Picospritzer, General Valve Co) to evoke the ACh potentials.

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Drug used were verapamil, 4–amino–pyridine, pyridostigmine bromide and $BaCl_2$(Sigma). Soman was obtained from the USAMRICD.

RESULTS

Effects of Ver on synaptic responses and ACh potentials In the SCG neurons of 10 rabbits and 6 guinea pigs, Ver (100–400 μmol / L) partially or completely depressed the synaptic responses (f–EPSPs and / or evoked action potentials) elicited by orthodromic stimulation and the ACh potentials induced by pressure ejection of ACh (Fig 1). The amplitude and duration of action potentials elicited by direct intracellular stimulation of the neuron appeared to be unchanged after application of Ver.

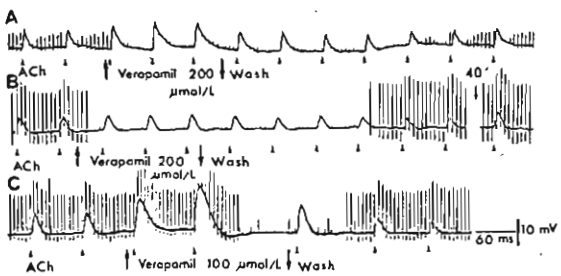

Fig 1. Effects of verapamil (Ver) on fast excitatory postsynaptic potentials (f–EPSPs) (A), action potentials (B, C) and acetylcholine (ACh) potentials in 3 rabbit superior cervical ganglion (SCG) cells.

Depression of synaptic responses occurred immediately after superfusion of Ver, while the depression of ACh potentials usually lagged behind that of the synaptic responses. Occasionally, an enhancement of ACh potentials occurred before the depressant effect. Further, the restoration of synaptic responses was not always parallel with that of ACh potentials. In most cases, the recovery of synaptic responses was faster than that of ACh potentials (Fig 1 B and C).

Effects of 4–AP and $BaCl_2$ on f–EPSPs and ACh potentials depressed by Ver 4–AP (100 μ mol / L) augmented the synaptic responses depressed by Ver. Either the amplitude of f–EPSPs increased (Fig 2 A) or the abolished synaptic responses reappeared after applicaiton of 4–AP. However, 4–AP had no notable effect on the ACh potentials. $BaCl_2$(5 mmol / L) facilitated the f–EPSPs depressed by Ver with a membrane depolarization, but the ACh potentials were not affected. The facilitation of f–EPSPs induced by $BaCl_2$ could still be found after the membrane potentials had repolarized to the resting level (Fig 2 B).

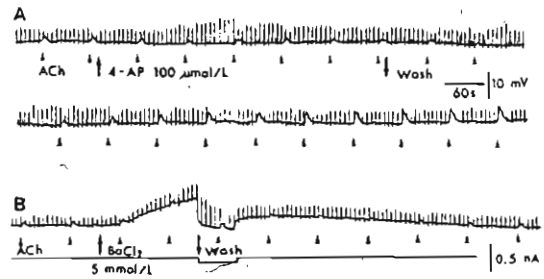

Fig 2. Effects of 4–aminopyridine (4–AP) and $BaCl_2$ on f–EPSPs and ACh potentials depressed by Ver in a rabbit SCG cell. This SCG was pretreated with Ver (100 μmol / L), so the amplitude of f–EPSPs diminished prior to application of 4–AP (A) or $BaCl_2$(B).

Effects of Ver on ACh potentials augmented by cholinesterase inhibitors Pyrido–

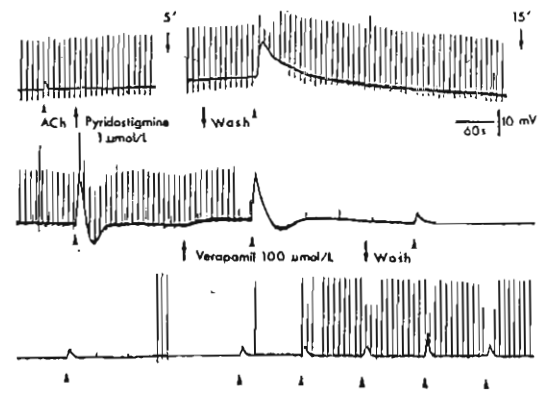

Fig 3. Effects of Ver on the ACh potentials augmented by pyridostigmine and on the synaptic responses in a rabbit SCG cell.

stigmine (PS, 1 μmol / L) greatly increased the amplitude and prolonged the duration of ACh potentials induced by exogenous ACh. Ver simultaneously depressed both the ACh potentials augmented by PS and the synaptic responses elicited by orthodromic stimulation (Fig 3).

The effect of soman (1 μmol / L) on ACh potentials was similar to that of PS as shown (Fig 3). After addition of Ver, the potentiating effect of soman on ACh potentials was prevented completely. The ACh potentials disappeared in spite of a sufficient concentration of soman being present (Fig 4).

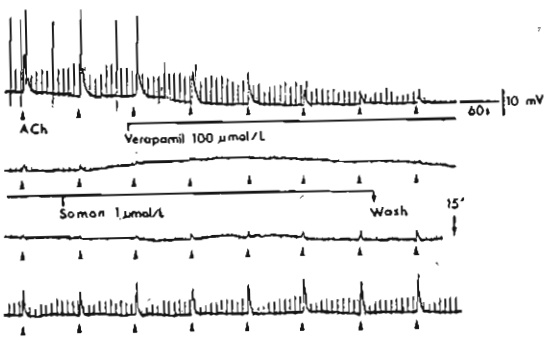

Fig 4. Effect of Ver on the response of guinea pig SCG cell to soman.

DISCUSSION

In the present study, Ver at higher concentrations (100–400 μmol / L) did not directly decrease the excitability of the mammalian SCG neurons since the action potentials elicited by direct intracellular stimulation of the cell were not affected by Ver. This is consistent with the results observed in mammalian skeletal muscle that Ver in the doses range of 0.01 – 1 mg / kg does not act directly on the muscle but on the neuromuscular junction[4].

The observations on the frog sartorius fibers indicated that Ver exerts a blocking effect on the nicotinic receptor via an allosteric mechanism[5]. Therefore, it is reasonable to assume that Ver can block the synaptic transmission in SCG with the mechanism similar to the effect of Ver on the neuromuscular junction.

It was demonstrated in this study that Ver exerted the depressant effects both on the synaptic responses elicited by orthodromic stimulation and the ACh potentials induced by pressure ejection of exogenous ACh in SCG neurons. The depression of the ACh potentials by Ver is probably postganglionic and produced by a decrease of the sensitivity of cholinergic receptors via an allosteric mechanism as in the neuromuscular junction. It has been observed in our recent work that soman (1–10 μmol / L) augmented the ACh potentials induced by exogenous ACh and its effect was long lasting [6]. Ver prevented such effect of soman and the similar effect of pyridostigmine. These findings support above assumption.

In the mammalian SCG, it has shown that 4–AP at lower concentrations (30–100 μmol / L) can facilitate the evoked release of ACh by a presynaptic mechanism[7], and that Ba^{2+} is more effective than Ca^{2+} in supporting the release of ACh by nerve impulses[8]. In the present study, the inhibitory effect of Ver on synaptic responses evoked by stimulating the preganglionic fibers was attenuated after application of 4–AP (100 μmol / L) or $BaCl_2$, while the ACh potentials induced by exogenous ACh was not affected. It therefore seems possible that Ver has effects on both the pre– and the postsynaptic sites. This is similar to the findings obtained from the neuromuscular junction that Ver can depress its transmission by both pre– and postjunctional mechanisms[9].

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维拉帕米对哺乳类交感神经节突触传递的影响

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提要 以细胞内微电极技术观察维拉帕米对离体颈上交感神经节(SCG)突触传递的影响.在家兔及豚鼠SCG神经元，维拉帕米(100-400 μmol/L)抑制顺向刺激引起的突触反应(快-兴奋性突触后电位(f-EPSP)和/或所诱发的动作电位)，以及压力加注ACh所产生的ACh电位.4-氨基吡啶(100 μmol/L)及氯化钡(5 mmol/L)可易化维拉帕米所抑制的f-EPSP，但对维拉帕米所抑制的ACh电位无明显作用.维拉帕米抑制吡啶斯的明(1μmol/L)所增强的ACh电位.索曼(1 μmol/L)对ACh电位的强化作用可被维拉帕米所阻抑.以上结果提示，维拉帕米可通过突触前及突触后作用，阻断哺乳类SCG的突触传递.

关键词 维拉帕米;交感神经节;氨基吡啶类;钡;溴化吡啶斯的明;索曼

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水飞蓟宾对缺血再灌注脑产生氧自由基、脂质过氧化物及白三烯的影响[1]

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Effects of silybin on production of oxygen free radical, lipoperoxide and leukotrienes in brain following ischmia and reperfusion[1]

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ABSTRACT Brain ischemia was produced by occluding bilateral common carotid arteries of Mongolia gerbil with clips for 30-min and reperfusion was established by removing the clips. Superoxide dismutase (SOD) in brain tissue was measured by chemoluminescence, malondialdehyde (MDA) by fluorescence spectrometry and leukotrienes (LT) by bioassay. Decrease of SOD and increase of MDA were significant in the brain after 30-min ischemia followed by 2-min reperfusion; The level of SOD increased from 13.4 ± 2.7 to 18.8 ± 3.0, 19.8 ± 2.5, 22.1 ± 3.9 × 10^3 units/g brain tissue and MDA decreased from 218 ± 26 to 169 ± 41, 167 ± 36, 167 ± 44 nmol/g brain tissue respectively by ip silybin 100, 200 and 400 mg/kg 30-min before occlusion. LT decreased from 3.1 ± 1.0 to 1.5 ± 0.4 ng/g brain tissue after ip

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