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Depolarization Induced by Substance P on Rat Stellate Ganglion Neurons
P物质对大鼠星状神经节细胞的除极化*

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Abstract The effects of neuropeptide substance P (SP) on the neurons of the isolated stellate ganglia of the rat were investigated by means of intracellular recording techniques. At the concentration of 1μm to 10μm, SP caused a low, monophersic depolarization in 28 out of 35 cells tested, low Ca^{2+} or tetrodotoxin (TTX) -containing Kreb's solution did not cause any significant change of the amplitude or duration of SP-induced depolarization. SP-induced depolarization was often associated with an increase in membrane resistance. Generally, the response was reversed on membrane potential of -80 mV to -100 mV. It is concluded that SP is excitatory to stellate ganglionic neurons and serves to augment impulse transmission through the neurons. These findings also suggest that a reduction of membrane K^+ conductance may underline the depolarization action of SP.

Key words substance P, stellate ganglion, intracellular recording

摘要 应用细胞内生物电记录技术,观察神经肽 P物质 (SP) 对大鼠星状神经节细胞的影响。SP在1μmol~10μmol或更高的浓度范围内,供试35个细胞,有28个细胞发生膜除极反应。用低钙 (0.25 mm) 或用含河豚毒素 (TTX, 1μmol) 克氏液灌流神经节,不影响 SP引起的除极反应的幅度和时程。SP引起除极反应的同时常伴有膜电阻增大。当膜电位增大时,除极化反应幅度变小,反转电位为-80 mV至-100 mV。研究表明,SP对部分星状神经节细胞具有兴奋作用,使通过这些细胞的信息传递增强;SP对细胞膜的除极作用是由于其引起细胞膜钾导降低所致。

关键词 P物质 星状神经节 细胞生物电记录
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In addition to the classical transmitters, i. e. Ach and r-aminobutyric acid, immunoreactivities to a number of peptides have been reported in the both of central and peripheral nervous system. Substance P (SP) is one of the most abundant neuropeptides in the nervous systems, including the sympathetic ganglia[1~4]. As already studied, SP has been detected in pre- and paravertabral sympathetic ganglia of the guinea pig and the rat. A Ca^{2+}-dependent release of immunoreactive SP from mesenteric ganglia was induced by high K^+. It was suggested that dorsal root ganglion cells were the origin of fibbers that release SP in the inferior mesenteric ganglia (IMG)[5,6]. Studies showed that application of SP mimicked the non-cholinergic EPSP (Ls-EPSP) in the mammalian IMG and the Ls-EPSP was markedly suppressed during SP-induced depolarization[5,7]. The activation of the peripheral sympathetic system by intrathecal SP supports the hypothesis that SP is a transmitter in the distinct cardiovascular and behavioral responses[8].

It has long been known that sympathetic neurons in the stellate ganglion regulate heart rate and contraction strength. In our morphology and electrophysiological studies, stellate ganglion neurons were found to receive a complex presynaptic input arising from the caudal sympathetic trunk and from T1 to T2 thoracic ramus and, in 16% of calls, from a cardiac nerve[9,10]. Is there a role of SP in the stellate ganglia?

1 Materials and Methods

Wistar rats of either sex, weighing 300 g~400g, age 3~4 months were used in the study. The procedure used for intracellular recording and stimulation from neurons of left and right stellate ganglion has been described[9~11]. The ganglia were superfused with a Krebs solution gassed with 95% O_2 and 5% CO_2. The temperature of the solution was maintained at about 34±0.5℃. Signals were amplified via an amplifier in bridge-mode which permitted current injection. At the same time, signals were displayed on oscillo-

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scope and Pen recorder. A constant current source was derived from a stimulator and square wave. The cell membrane potential could be varied by injection of continuous D. C. current. The drugs (Sigma) were dissolved in Kreb's solution and applied to the ganglia by superfusion in known concentrations. SP (Sigma) was applied to the ganglion cells by pressure ejection from micropipette containing 0.1 mm SP. The peptide was ejected onto the ganglion cells from the micropipetter using a constant pressure (40 Psi) but variable duration (10 ms~ 990 ms) of nitrogen gas under visual control. The figures were reproduced from the tracings of a pen recorder. Numerical results are expressed as means standard error of the mean ($x \pm s$).

2 Results

2.1 Effect of SP

Results were obtained from stable recording stellate ganglion neurons. In 28 out of 35 cells tested. SP induced slow depolarization. The amplitude of depolarization varied from a few millivolts to 15 mV, the mean was 6.0± 0.8 mV when recorded at the membrane potential of − 50 to − 65 mV. The duration of SP-induced depolarization ranged from 1 to 5 minutes with a mean of 2.8± 0.8 minuets. Spike discharges were frequently seen during the rising or plateau phase of SP-induced depolarization (Figs. 1, 2)

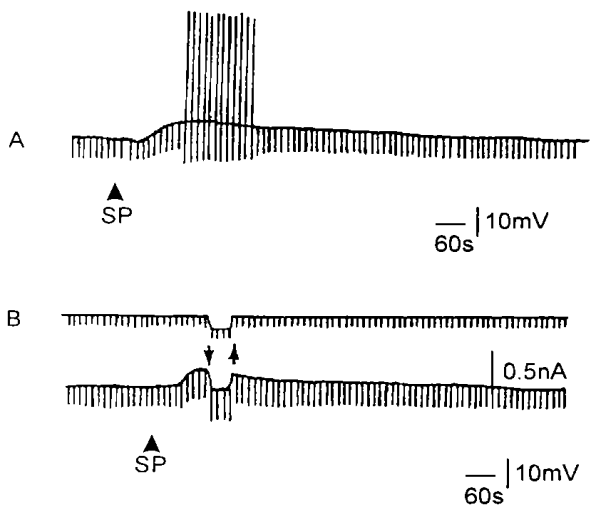

Fig. 1 SP elicited a depolarization and increased input resistance. At the peak of the depolarization, membrane potential was manually restored to the resting level (indicated by two arrows) (B), under this condition there was a 20% increase in membrane input resistance. A, B were taken from the same cell.

2.2 Effects of low Ca^{2+} and TTX

Superfusing the ganglia with low Ca^{2+} (0.25 mm), high Mg^{2+} solution (12 mm, n = 5) or TTX (1μm, n = 4), containing Kreb's solution did not cause any significant change of the amplitude or duration of SP-induced depolarization (Fig. 3).

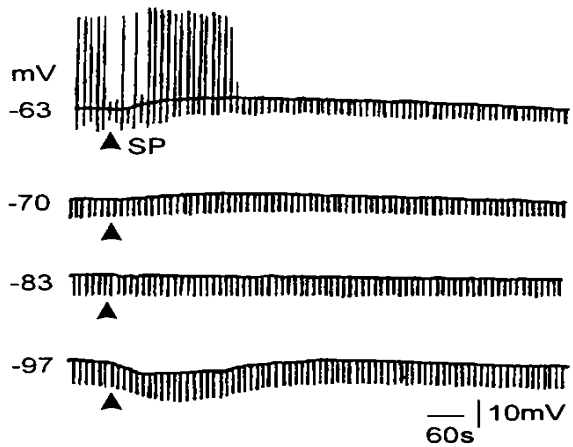

Fig. 2 Reversal of SP-induced depolarization by membrane hyperpolarization. At a membrane potential of − 97 mV, SP caused a hyperpolarization instead of a depolarization.

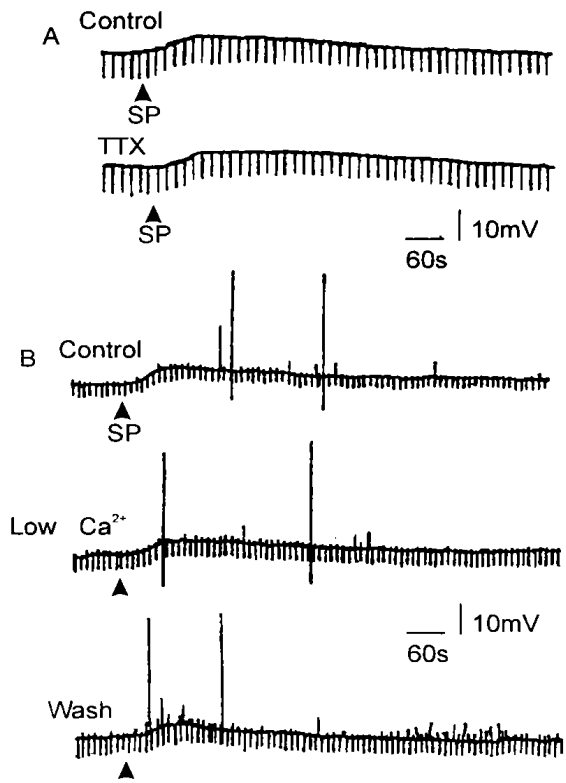

Fig. 3 Lack of effect of TTX or Low Ca^{2+} /high Mg^{2+} on SP-induced depolarization of neurons. SP was applied at arrowheads by ejection pulses. Superfusion of TTX and low Ca^{2+} / high Mg^{2+} solution lasted 15 (A) and 20 minutes (B) respectively.

2.3 Reduction of K^+ conductance

The membrane input resistance as monitored by the amplitude of hyperpolarizing electronic potentials showed either no change or a slight increase during SP-induced depolarization of the stellate ganglion neurons. The slow time course of the depolarization made it possible to clamp manually the membrane potential during the response. Under these conditions, a clear increase in membrane resistance was observed in 10 of

13 cells tested (increased 21% 8%) (Fig. 1). In the remaining three neurons, the membrane resistance showed no measurable change.

The amplitude of the SP-induced depolarization was inversely related to membrane potential in 8 of 10 cells studied. The response was made smaller on membrane hyperpolarization and it was made smaller on membrane potential of − 80 mV to − 100 mV (91.2± 3.5 mV, n = 8). A representative experiment is shown in Fig. 2. It should be noted, in 2 cells, membrane hyperpolarization increased the amplitude of SP-induced depolarization. In another 2 cells, the response became smaller on membrane hyperpolarization and a clear reversal was not seen.

3 Discussion

The principal observation made in this study is that SP depolarized and excited stellate ganglion neurons. It is noteworthy that the characteristics of SP-induced depolarization in stellate ganglion neurons, namely a considerable delay at the onset, the slow rise and slow decay of the response and the induction of cell discharge, are similar to the characteristics described for the SP response of other central and peripheral neurons that have been studied[7, 12, 15]. Furthermore, the observation that SP-produced effect was not appreciably affected by low Ca^{2+}, nor by TTX, suggests that the peptide depolarizes ganglion cell by a direct action, is not via a release of acetylcholine or other endogenous substances.

The SP-induced depolarization of stellate ganglion neurons was associated with an increase in memberane resistance, it was reduced by hyperpolarization, and it was reversed at the membrane potential of − 80 mV and − 100 mV. All these findings suggest that a reduction of membrane K^+ conductance constitutes the primary ionic mechanism underlying the depolarizing actions of this peptide. A similar ionic mechanism postulated for the depolarizing actions of SP on mammalian central and peripheral neurons[2, 13]. However, in 2 cases the depolarization was increased or no change membrane hyperpolarization, and while in a few neurones, the response was decreased by hyperpolarization, it was not reversed. These findings suggest that other ionic specied, e. g. Na^+ and /or Ca^{2+}, in addition to K^+, may have been affected, as has been suggested the SP response of the guinea-pig inferior mesenteric ganglion cells[7], rat dorsal horn[14] and lateral horn cells[15]. On the other hand, the reductions of M-current and an inwardly rectifying K^+ current have been shown to underlie the depolarizing action of SP on bull-frog sympathetic neurons and rat cultured megnocellular neurons, respectively[12].

Is there a physiological significance of the present findings? SP-induced depolarization brings the cell closer to the threshold level and increases membrane resistance. The physiological consequence of SP-induced depolarization at stellate ganglion neurons may serve to provide a temporal and spatial mechanism whereby the likelihood of spike discharge of the target cell is markedly potentiated, leading to an increase in vasomotor activity.

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