BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1997 Mar; 18 (2): 124 - 127

Adrenoceptor agonists inhibit calcium-dependent potentials in rat stellate ganglion neurons[1]

MO Ning, LI Dong
(*Department of Pharmacology, Guangxi Medical College, Nanning 530021, China*)

KEY WORDS stellate ganglion; electric stimulation; action potentials; calcium; clonidine; adrenergic alpha-antagonists; tetrodoxin; tetraammonium compounds

AIM: To study the effects of adrenoceptor agonists on the stellate ganglion neurons. **METHODS**: Intracellular recordings were made from neurons of the isolated rat stellate ganglia. **RESULTS**: Noradrenaline and clonidine (10 - 30 $\mu mol \cdot L^{-1}$) reversibly depressed 3 types of calcium-dependent potentials, namely, the action potential shoulder; the spike after-hyperpolarization; the Ca^{2+} spike evoked in Krebs' solution containing TTX and TEA and fast excitatory postsynaptic potential (f-EPSP). **CONCLUSION**: The adrenoceptor agonists inhibitied the 3 calcium-dependent potentials; f-EPSP was inhibited by reducing Ca^{2+} influx at presynaptic site in population of neurons.

At interneuronal junctions, catecholamines seem to regulate the excitability of target cells by modifying their resting membrane potentials and conductance or by producing postsynaptic potentials. In the case of amphibian sympathetic ganglion cells, these cell membranes are depolarized or hyperpolarized by the action of catecholamines[1] and the slow inhibitory postsynaptic potential (s-IPSP) of these cells seems to be produced by the action of catecholamines[2]. In bullfrog sympathetic ganglion cells, catecholamines regulate the configuration of action potentials[3] and have a direct facilitatory effect on acetylcholine release[4]. Catecholamines exerted a biphasic effect on the noncholinergic excitatory postsynaptic potential (noncholinergic epsp) of the inferior mesenteric ganglion cells[5]. The purpose of the present study was to investigate the effects of catecholamines on the isolated stellate ganglion of the rat.

[1] Project supported by the National Natural Science Foundation of China (No 39470242) and the British Heart Foundation.
Received 1996-07-30 Accepted 1996-11-22

MATERIALS AND METHODS

Wistar rats of either sex (3 - 4 months) were killed. The stellate ganglia with their sympathetic nerve trunks were superfused with the Krebs' solution gassed with 95 % O_2 + 5 % CO_2 at 34 ± 0.5 ℃. The ganglion was stimulated either antidromically by the postganglionic nerves or orthodromically by preganglionic nerve branches with a suction electrode. Intracellular recordings were obtained by fiber-containing glass microelectrodes filled with KCl(3 $mol \cdot L^{-1}$), which had resistances of 30 - 60 MΩ. The input resistance of the cells was calculated from voltage changes produced by passing repetitive hyperpolarizing current pulses. Signals were amplified *via* an Axoclamp-2A amplifier. Signals were displayed on oscilloscope and pen recorder[6-8].

(-)-Noradrenaline bitartrate, clonidine hydrochloride, idazoxan hydrochloride, isoprenaline hydrochloride, phentolamine hydrochloride, prazosin hydrochloride, and yohimbine hydrochloride were purchased from Sigma Co. The drugs were dissolved in Krebs' solution to superfusion.

The results are expressed as $\bar{x} \pm s$. Statistical difference was evaluated by t-test.

RESULTS

Effects on 3 Ca^{2+} dependent potentials

Three voltage responses in stellate postganglionic neurons which are Ca^{2+} dependent have been identified. The spike after-hyperpolarization (AH) and a "shoulder" were seen during discharge in normal Krebs' solution ($n = 40$). Both the shoulder and the AH decreased in magnitude as extracellular Ca^{2+} concentration was lowered from the normal level of 2.5 to 0.25 $mmol \cdot L^{-1}$. The third Ca^{2+}-dependent potential is the spike potential evoked in Krebs' solution containing tetrodotoxin (TTX, 1 $\mu mol \cdot L^{-1}$) and TEA (10 $mmol \cdot L^{-1}$) ($n = 6$).

Noradrenaline and clonidine reversibly reduced the action potential shoulder and magnitude of AH in 22 out of 30 neurons studied. Noradrenaline and clonidine attenuated the amplitude of AH from 9.5

± 1.0 mV to 7.3 ± 0.7 mV ($n = 12$, $P < 0.05$) and reduced the action potential shoulder by 56.5 ± 1.3 % ($n = 12$, $P < 0.05$). These effects occurred after 1 – 2 min of exposure to drugs did not desensitize over 5 min period, and were reversed within 2 min of washing (Fig 1A). The actions of noradrenaline and clonidine on the Ca^{2+}-dependent potentials were mimicked by low Ca^{2-} (Fig 1B).

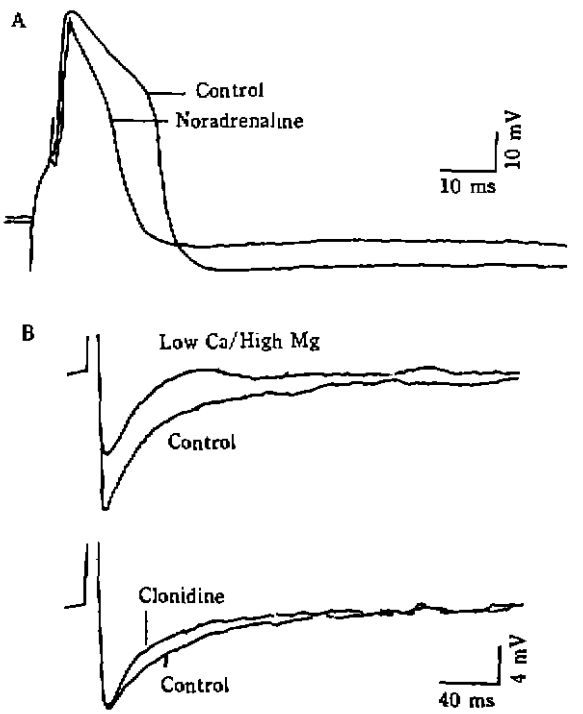

Fig 1. Effects on AH in 2 cells. A. Superfusion with noradrenaline. B. Superfusion with a low Ca^{2+}/high Mg^{2+} solution and clonidine which were recorded from the same cell.

To ascertain the effect of these adrenoceptor agonists on calcium spikes, some neurons were superfused with Krebs' solution containing TTX (1 μmol·L^{-1}) and TEA (10 mmol·L^{-1}). Noradrenaline reversibly diminished both the rate of rise and the duration of Ca^{2+} spike in 6 tested cells. Measured from 13 Ca^{2+} spikes, the mean spike half width (duration at 50 % peak amplitude) was 37 ± 4 ms before and 31.6 ± 1.3 ms ($P < 0.05$) after and 10 % – 90 % rise time was 6.5 ± 0.2 ms before and 6.9 ± 0.1 ms ($P < 0.05$) after superfusion with noradrenaline. The depressant effect of noradrenaline was reversible by 2 – 3 min wash with drug-free Krebs' solution. Noradrenaline depressed the Ca^{2+} spike without affecting the membrane potential and input resistance (Fig 2).

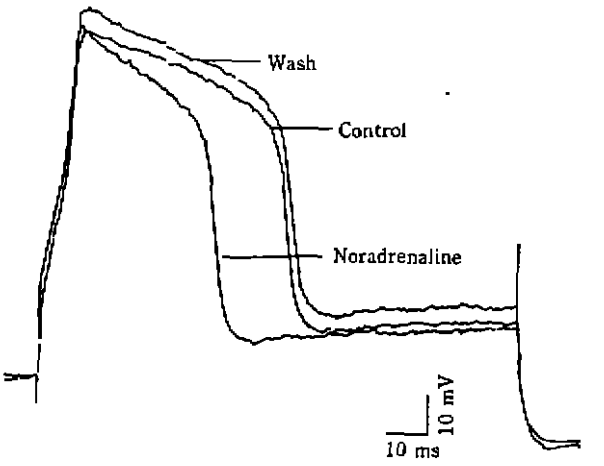

Fig 2. Depression of Ca^{2+} action potential by noradrenaline in a neurone. The ganglion was pretreated and superfused with TTX plus TEA.

Effects of α- and β-adrenoceptor antagonists

The depressant actions of noradrenaline and clonidine on the Ca^{2-}-dependent potentials were prevented by α-adrenergic antagonist idazoxan (1 – 10 μmol·L^{-1}, $n = 5$), which *per se* did not change the resting membrane potential and input resistance. Superfusion of idazoxan effectively antagonized the depressant effects of noradrenaline on action potential shoulder and AH (Fig 3). Propranolol (10 μmol·L^{-1}), a β-adrenergic antagonist,

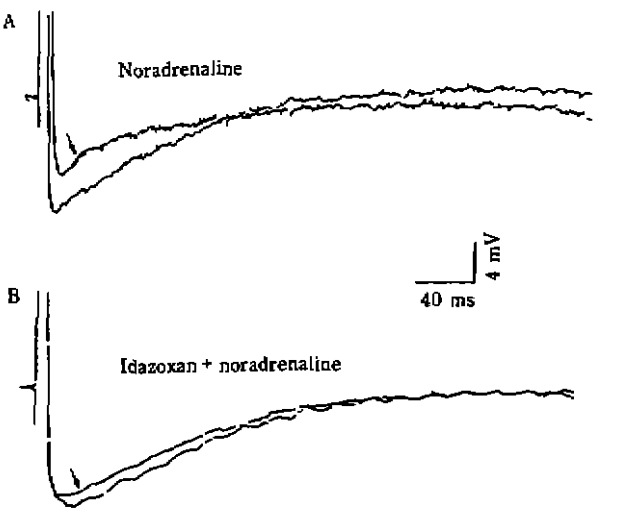

Fig 3. Attenuation of noradrenaline depression of AH by idazoxan. Superfusion with noradrenaline (A) or idazoxan (B) on the same cell.

was without effect ($n = 3$). These findings suggest that noradrenaline and clonidine act through α-adrenergic receptor. Pre-treatment of the ganglia with phentolamine ($n = 6$), prazosin ($n = 5$), and yohimbine ($n = 3$) at the concentrations of 1 − 10 $\mu mol \cdot L^{-1}$ for 25 − 30 min failed to antagonize the effects of noradrenaline or clonidine.

Effects on f-EPSP In addition to depressing the Ca^{2+}-dependant potentials, at the concentrations of 10 − 30 $\mu mol \cdot L^{-1}$, when applied to ganglia of 2 − 5 min, noradrenaline and clonidine, which caused no significant change of resting membrane potentials, reversibly depressed or completely blocked the f-EPSP in 7 out of 10 cells examined. The depressant effect was reversed after 20 min period of washing with Krebs' solution free of drug (Fig 4).

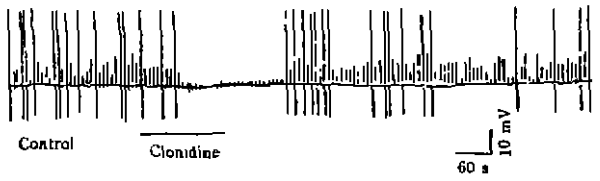

Fig 4. Effect of clonidine on f-EPSP evoked by preganglionic nerve stimulation in a neurone. Stimulation elicited threshold potentials (small upward deflections) and spike potentials (larger upward deflections). Clonidine depressed the synaptic responses.

DISCUSSION

A hypothesis to account for these observations is the adrenoceptor agonists inhibit an inward Ca^{2+} current. Previous studies suggested that AH was mediated principally by an increase of G_K secondary to Ca^{2-} influx[9,10]. In this regard, a more direct assessment of adrenoceptor agonists on Ca^{2+} conductance was obtained with respect to the spike potentials evoked in TTX and TEA containing Krebs' solution. Under this condition, the effective abolition of the Ca^{2+} spike supports the contention that adrenoceptor agonists reduce Ca^{2+} conductance rather than the G_K (Ca)[9,11,12]. It has been reported catecholamines antagonize synaptic transmission in mammalian sympathetic ganglion by an action at presynaptic site through an α-adrenoceptor and reduction in neurotransmitter release[13-15]. the experiment showed that adrenaline and clonidine depressed f-EPSP. This presynaptic action is to reduce Ca^{2+} influx into the terminal, which results in reduced transmitter release.

The findings that Idozoxan but not pentalamine or prazosin antagonized the depressant effects of adrenoceptor agonists are consistent with the idea that the depressions were mediated by α_2-adrenergic receptors. However, in 3 cases the effects of noradrenaline and clonidine were not significantly changed by yohimbine (α_2-adrenergic antagonist). The possibility for the discrepancy may have following explanations. First, in stellate ganglion cells, receptors were more sensitive to idozoxan than yohimbine; Secondly, other adrenergic receptor sub-types, in addition to α_2-adrenergic receptors mediated the effects of the agonists. This implied a selective distribution of adrenergic receptors to a subpopulation of stellate ganglion neurons. The question of the type of α-adrenergic receptor that responsed the effects of noradrenaline in the stellate ganglia remains to be explored using more specific antagonists that are capable of differentiating subtypes of α-adrenergic receptors.

Is there a physiological significance of the present findings? It is to point out here that mammalian sympathetic ganglia contain a relatively large number of small intensely fluorescent cells, the amine contents of which were mostly noradrenaline and adrenerline. The question of whether or not catecholamines released from these intensely fluorescent cells under physiological conditions and served a role similar to that described in the present study needs to be ascertained.

REFERENCES

1 Nakamura M, Koketsu K. The effect of adrenaline on sympathetic ganglion cells of bullfrogs
Life Sci 1972; (Pt 1) 11: 1165 − 73.

2 Libet B, Kobayashi H. Adrenergic mediation of slow inhibitory postsynaptic potential in sympathetic ganglia of the frog.
J Neurophysiol 1974; 37: 805 − 14

3 Minota S, Koketsu K. Effects of adrenaline on the action potential of sympathetic ganglion cells in bullfrogs.
Jpn J Physiol 1977; 27: 353 − 66.

4 Kuba K, Kato E, Kumamoto E, Koketsu K, Hirai K.
Sustained potentiation of transmitter release by adrenaline and

dibutyryl cyclic AMP in sympathetic ganglia
Nature 1981; 291: 654 - 6.
5 Dun NJ, Jiang ZG, Mo N Long-term facilitation of peptidergic transmission by catecholamines in guinea-pig inferior mesenteric ganglia. J Physiol (Lond) 1984; 357: 37 - 50.
6 Mo N, Wallis DI, Aatsen A Putative cardiac and non-cardiac neurones in the rat stellate ganglion.
J Auton Nerv Syst 1994; 47: 7 - 22.
7 Mo N. Facilitation of nicotinic transmission in guinea pig isolated superior cervical ganglion cells by cobrotoxin.
Chin J Pharmacol Toxicol 1989; 3: 192 - 6
8 Mo N. Depolarization induced by and calcium-dependent after-hyperpolarization suppressed by muscarine in rat stellate ganglion neurons. Chin J Pharmacol Toxicol 1996; 10: 12 - 6.
9 McAfee DA, Yarowsky PJ. Calcium-dependent potentials in the mammalian sympathetic neurone.
J Physiol (Lond) 1979; 290: 507 - 23.
10 Busis NA, Weight FE. Spike after-hyperpolarization of a sympathetic neurone is calcium sensitive and is potentiated by theophylline Nature 1976; 263: 434 - 6.
11 Hashiguchi T. The calcium-dependent components of action potential of the rabbit superior cervical ganglion cells.
J Tokyo Med Coll 1979; 37: 533 - 44.
12 Mo N, Ammari R, Dun NJ. Prostaglandin E_1 inhibits calcium-dependent potentials in mammalian sympathetic neurons.
Brain Res 1985; 334: 325 - 9.
13 Christ DD, Nishi S Site of adrenaline blockade in the superior cervical ganglion of the rabbit
J Physiol (Lond) 1971; 213: 107 - 17.
14 Dun NJ, Nishi S. Effects of dopamine on the superior cervical ganglion of the rabbit.
J Physiol (Lond) 1974; 239: 155 - 64.
15 Dun NJ, Karczmar AG. The presynaptic site of action of norepinephrine in the superior cervical ganglion of guinea-pig.
J Pharmacol Exp Ther 1977; 200: 328 - 35

肾上腺素受体激动剂抑制大鼠星状神经节细胞钙依赖性电位

莫 宁, 李 东
(广西医科大学药理教研室, 南宁 530021, 中国)

关键词 星状神经节; 电刺激; 动作电位; 钙; 可乐定; 肾上腺素 α-拮抗剂; 河鲀毒素; 季铵化合物

目的: 探索肾上腺素能受体激动剂对大鼠星状神经节细胞的作用及其作用机制. **方法**: 离体交感神经节细胞内生物电记录. **结果**: 去甲肾上腺素, 可乐定($10-30\ \mu mol\cdot L^{-1}$)可逆地抑制细胞动作电位; 依钙后超极化电位; 在含 TTX 与 TEA 克氏液中记录到的钙锋电位及快兴奋性突触后电位. **结论**: 肾上腺素受体激动剂对三种钙电位及快兴奋性突触后电位的抑制作用可能是其在突触前膜对钙内流的抑制所致.