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Modulation of neuronal nicotinic acetylcholine receptors by glucocorticoids

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SHI Li-Jun[1], LIU Ling-Ai, CHENG Xiao-Hong, WANG Chun-An[2]
(*Department of Physiology, Beijing Medical College of PLA, Beijing 100071*; [2]*Department of Physiology, Second Military Medical University, Shanghai 200433, China*)

KEY WORDS glucocorticoids; nicotinic receptors; patch-clamp techniques

ABSTRACT

AIM: To investigate the nongenomic effect of the glucocorticoid corticosterone on nicotinic acetylcholine receptor (nAChR) in PC12 cells. **METHODS**: The acetylcholine (ACh)-induced current was measured on nerve growth factor differentiated PC12 cells using whole-cell patch-clamp techniques. **RESULTS**: The ACh-induced current (I_{ACh}) proved to be generated through neuronal nAChR. When ACh (30 μmol/L) was applied simultaneously with corticosterone (0.1 - 10 μmol/L), the decay of I_{ACh} was faster with slight inhibition on the peak current amplitude. Pretreating PC12 cells with corticosterone augmented the inhibition on the peak I_{ACh} and did not alter receptor desensitization. Bovine serum albumin-conjugated corticosterone (0.1 - 10 μmol/L) had the inhibition similar to corticosterone. The rapid effect induced by corticosterone was reversible, concentration-dependent, and voltage-independent. **CONCLUSION**: Corticosterone has rapid inhibitory effect on I_{ACh}, which is mediated by a nongenomic mechanism. It indicates that corticosterone binds to the specific site on the outer cell membrane, probably on the neuronal nicotinic receptor-coupled channel, and inhibits the I_{ACh} in a noncompetitive manner, thus controlling the immediate catecholamine release from the sympathetic cells.

INTRODUCTION

Evidence for nongenomic actions of steroids is now coming from a variety of fields of steroid research. For example, in recent years, a number of studies have shown that steroids can have a rapid action in the brain and can produce clinical anesthesia as well as affecting mood and performance in humans. These rapid actions are apparently mediated by binding to membrane proteins, including ligand-gated ion channels. A variety of steroids can enhance activation of the γ-aminobutyric acid type A ($GABA_A$) receptor by GABA and may cause direct gating of this receptor[1]. Certain sulfated steroids block $GABA_A$ receptors[2] and can also enhance the activity of *N*-methyl-*D*-glutamate-type glutamate receptors[3]. Finally, steroids are known to inhibit the muscle[4,5] and neuronal[6-8] nicotinic receptor. But little is known about the modulation of the natural glucocorticoids on neuronal nicotinic acetylcholine receptor (nAChR) in sympathetic cells. When cultured in the presence of nerve growth factor (NGF), pheochromocytoma (PC12) cells differentiated to resemble sympathetic neurons morphologically and functionally[9]. The aim of the present study was to investigate the short-term effect of corticosterone, a natural glucocorticoid of rat, on the electrical excitability and ACh-induced current (I_{ACh}) in PC12 cells.

MATERIALS AND METHODS

Cell culture PC12 cells grew in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10 % fetal bovine serum and 5 % horse serum (Gibco). Cells were plated onto collagen-coated cover-slips which were placed in 35 mm plastic tissue culture dishes and treated with 2.5S-NGF at 50 μg/L. The cultures were fed three times a week and were used for electrophysiological experiment 5 - 10 d after being plated.

Whole-cell patch clamp recording Cover-slips bearing PC12 cells were removed from culture medium and placed into the recording chamber (volume

[1] Correspondence to Dr SHI Li-Jun. Phn 86-10-6694-7680. Fax 86-10-6278-4691. E-mail l.j.shi@263.net
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approximately 0.6 mL) which was perfused continuously with extracellular solution containing NaCl 140, KCl 5, $CaCl_2$ 2.5, $MgCl_2$ 1, HEPES 10, glucose 11.5 mmol/L, pH 7.4. The basic patch electrode-filling solution included KCl 140, egtazic acid 0.5, HEPES 5, $MgCl_2$ 1, $CaCl_2$ 0.397, NaOH 3, ATP-2Na 1 mmol/L, pH 7.2 – 7.3. Recording was obtained from isolated cells lacking processes using standard patch clamp techniques[10]. Patch-recording electrodes were pulled from borosilicate glass micropipettes on a PC-10 puller (Narishige, Japan). Electrode resistance in recording solution was 2 – 5 MΩ. Membrane currents from whole-cell voltage clamp were recorded using a CEZ-2300 amplifier (Nihon Kohden, Japan) and were fed to a pen recorder (LMS-2B, Chengdu, China, frequency response 5 Hz at – 3 dB). Chemicals were diluted in the extracelullar perfusing solution. External solutions were changed by gravity feed and flow was controlled via some three-way valves fixed in proximity to the bath. The solution changing time was minimized to 3 – 8 s. Cells were voltage-clamped to – 80 mV unless otherwise stated. All experiments were performed at room temperature (22 ℃ – 24 ℃).

Statistical analysis Data were expressed as $\bar{x} \pm s$. Paired t test was used for testing the significance between the peak amplitude of I_{ACh} before and after perfusion the test drug. ANOVA was used for testing the significance among the inhibition rates of corticosterone at various concentrations or holding potentials.

RESULTS

I_{ACh} in PC12 cells PC12 is a clonal cell line derived from a catecholamine-secreting tumor (pheochromocytoma) of rat adrenal medulla. When cultured in the presence of NGF, PC12 cells differentiate to resemble sympathetic neurons morphologically, accompanied by physiological and biochemical changes which ultimately endow PC12 cells with neuron-like function[9]. After the membrane was ruptured, the membrane potential was held at – 80 mV. Bath application of ACh 30 μmol/L produced an inward current associated with an increase in current noise, a reflection of stochastic behavior of the activated channels. The peak amplitude of I_{ACh} was (0.62 ± 0.11) nA (n = 24). The I_{ACh} proved to be generated through neuronal nAChR, as had been reported in our previous work[11]. When ACh was continuously present in the bath for 40 s, the current amplitude gradually decreased to a sustained level due to the desensitization process (Fig 1). To ensure recovery from desensitization, an interval of at least 2 min between repetitive applications of ACh was used[12].

Rapid inhibition by corticosterone on I_{ACh} in PC12 cells Corticosterone 21-sulfate (Sigma) had no effect on the resting membrane conductance (Fig 1). When voltage clamped at – 80 mV, ACh 30 μmol/L was applied simultaneously with corticosterone 10 μmol/L. As shown in Fig 1a, the peak amplitude of I_{ACh} was inhibited. The rate of inhibition was 15.2 % ± 4.8 % (n = 8). This inhibition of I_{ACh} by corticosterone was reversible and was easily eliminated by its washout (Fig 1a). When cells were pretreated with corticosterone 0.01 – 100 μmol/L for 4 min, the peak amplitude of the subsequently elicited I_{ACh} in the presence of corticosterone was greatly inhibited (Fig 1b, Fig 2).

The relationship between inhibition and the concentration of corticosterone is shown in Fig 2. The inhibition data were fitted with the equation:

$$Y = 1/\{1 + ([C]/IC_{50})^n\} \quad (1)$$

where IC_{50} is the concentration of steroid (C) producing half-reduction in the response and n is the Hill coefficient. The fit parameters for the data in Fig 2 (responses elicited with ACh 30 μmol/L) were an IC_{50} (95 % confidence limits) value of 12.5 (11.3 – 13.6) μmol/L and an n value of 0.6.

Cholesterol (0.01 – 100 μmol/L) had no effect on I_{ACh} (Fig 1c). Furthermore, inhibition by corticosterone was not affected by the concomitant application of cholesterol (10 μmol/L, n = 6) (data not shown). Bovine serum albumin-conjugated corticosterone (B-BSA) is a water-soluble compound that does not enter the plasma membrane. Pretreating cells with B-BSA (0.1 – 10 μmol/L) for 4 min had the inhibition on I_{ACh} similar to corticosterone (Fig 1d). It was also concentration-dependent. BSA alone had no detectable effects on the I_{ACh} when applied before or during the ACh application (Fig 1e).

The effect of corticosterone on the desensitization of I_{ACh} in PC12 cells The decay of 30 μmol/L ACh-induced current in the controls could be fitted by a single exponential function in most cases (5 of 6 cells) with time constants of 11.3 s ± 0.6 s (τ). Simultaneous application of corticosterone 10 μmol/L and ACh 30 μmol/L greatly facilitated the falling phase of the

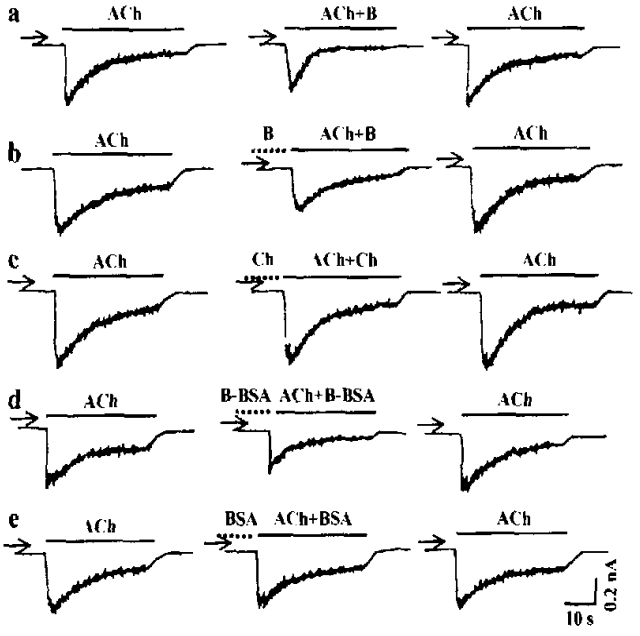

Fig 1. Rapid inhibition of I_{ACh} in PC12 cells by extracellular application of corticosterone (B), cholesterol (Ch), corticosterone-conjugated BSA (B-BSA) and BSA. Simultaneous application of B and ACh to a PC12 cell (a); Pretreatment with B (b), Ch (c), B-BSA (d), BSA (e) for 4 min respectively (dotted line), and then application of ACh and the corresponding substance simultaneously. Bars indicate periods of applications. Holding potential: −80 mV. ACh: 30 μmol/L; B, Ch, B-BSA, and BSA: 10 μmol/L. Arrows indicate the zero current level.

I_{ACh} (Fig 1a), which consisted of two exponential components with time constants of 3.6 s ± 0.7 s (τ_f), 10.3 s ± 1.4 s (τ_s) ($n = 5$). The effect was also reversible. By statistical analysis, it was found that τ_s was comparable to τ of the control value ($P > 0.05$). When the cell pretreated with corticosterone (10 μmol/L) for 4 min, then applied with ACh and corticosterone simultaneously, we found the desensitization of I_{ACh} had no noticeable change (Fig 1b). There was a single exponential time course observed, with a time constant of 10.9 s ± 0.8 s ($n = 5$). This value was comparable to the control value ($P > 0.05$).

Inhibition by corticosterone is not competitive To further clarify the mode of inhibition, the effects of corticosterone on the concentration-response relationship of I_{ACh} were examined (Fig 3). The best fit for the control peak I_{ACh} was made with a theoretical line when it was assumed that the dissociation constant (K_d) value 16.5 μmol/L and the Hill coefficient was 1.8. The data points for the inhibition of I_{ACh} induced by corticosterone (10 μmol/L) in the cells pretreated with corticosterone for 4 min showed a good fit with the same value of each parameter except for 49 % reduction of the maximal response. From this result, it is very likely that corticosterone inhibits the I_{ACh} in a noncompetitive manner.

Inhibition by corticosterone is voltage-independent To determine the influence of the membrane potential on the inhibition by corticosterone on I_{ACh}, the current-voltage relationship of responses to ACh was firstly recorded under control conditions and then in the presence of corticosterone 10 μmol/L after cells were pretreated with corticosterone for 4 min (Fig 4). It could be seen that corticosterone had rapid inhibition on I_{ACh} at various holding potentials from −80 to −40 mV, and there was significant difference between the peak currents before and after pretreatment with corticosterone ($P < 0.01$). However, ANOVA showed no significant differences among the rates of the inhibition ($P > 0.05$). This result indicates that the inhibition by corticosterone on I_{ACh} is voltage-independent.

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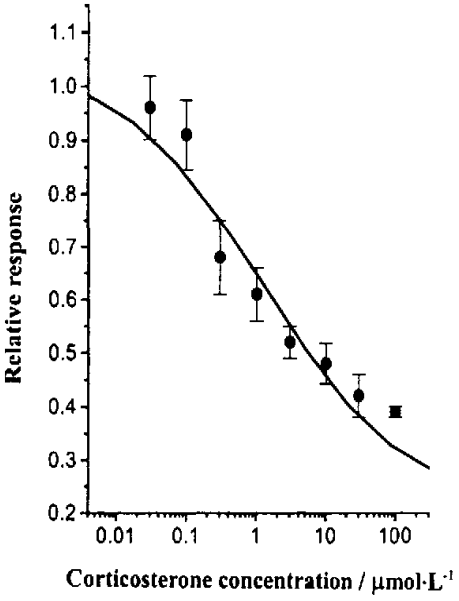

Fig 2. Corticosterone concentration-response inhibition of I_{ACh}. The peak amplitude of I_{ACh} was normalized with respect to the value recorded when using ACh 30 μmol/L alone. Holding potential: −80 mV. $n = 6$. $\bar{x} \pm s$.

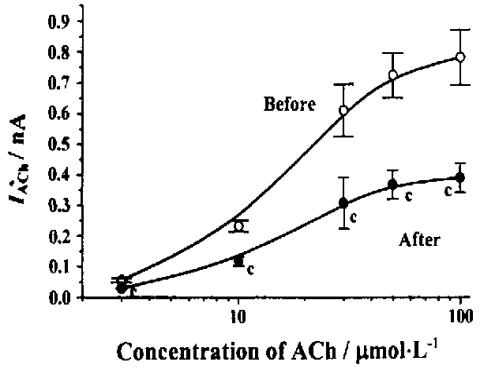

Fig 3. Effect of corticosterone on concentration-response relationships of I_{ACh}. Peak magnitudes of current in cells were plotted against ACh concentrations. (○, ●) Before and after pretreatment with corticosterone 10 μmol/L for 4 min. Data of control I_{ACh} and I_{ACh} inhibited by corticosterone (10 μmol/L) were well fitted to each theoretical line: $I = I_{max}(C^n)/(C^n + K_d^n)$, where I is observed peak I_{ACh}, I_{max} is maximal value of peak I_{ACh} with (0.40 nA) and without (0.82 nA) corticosterone, C is ACh concentration, K_d is a constant corresponding to concentration of ACh producing a half-maximal current (16.5 μmol/L), and n is the Hill coefficient. $n = 6$. $\bar{x} \pm s$. $^cP < 0.01$ *vs* the peak amplitude of I_{ACh} before pretreatment with corticosterone.

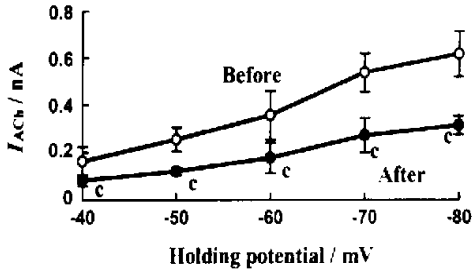

Fig 4. Inhibition by corticosterone on I_{ACh} in PC12 cells at various holding potentials. The cells were incubated with corticosterone 10 μmol/L for 4 min at various holding potentials, and then were applied with corticosterone 10 μmol/L and ACh 30 μmol/L simultaneously. (○, ●): Before and after pretreatment with corticosterone for 4 min. $n = 6$. $\bar{x} \pm s$. $^cP < 0.01$ *vs* the peak amplitude of I_{ACh} before pretreatment with corticosterone.

DISCUSSION

When cultured in the presence of NGF, PC12 cells differentiate to resemble sympathetic neurons morphologically and functionally. The ACh-induced current in PC12 cells proved to be generated through the nicotinic acetylcholine receptor, which was in agreement with the report of Boyd[13]. In present study, we found corticosterone, the natural glucocorticoid of rat, could inhibit I_{ACh} rapidly. The rapid onset and short-time course of the inhibition by corticosterone on I_{ACh} suggests that it is distinct from the genomic mechanism of action and is a nongenomic effect. As we know, glucocorticoids are synthesized from cholesterol in the adrenal gland. The mode of action of cholesterol has been attributed to an alteration of the lipid composition of the plasma membrane surrounding the nAChRs[14]. The lack of action of cholesterol on nAChR of PC12 cells indicates that corticosterone inhibition is unlikely to occur via a membrane perturbation but rather reflects a direct action on the receptor. B-BSA is a large molecule and a water-soluble compound that does not enter the plasma membrane, while it was effective in reducing the I_{ACh}. This result indicates that corticosterone acts via the extracellular compartment.

Application of corticosterone consistently inhibited the I_{ACh} in a noncompetitive manner. The magnitude of the inhibition was little changed over a range of ACh concentrations from 3 to 100 μmol/L. Therefore, it is probable that corticosterone suppressed the I_{ACh} with no

effect on the apparent affinity of ACh for its receptor complex. Another possible mode of corticosterone inhibition of the I_{ACh} is open channel blocker (OCB), which has been demonstrated with various kinds of chemical[15]. In general, OCBs are strongly voltage-dependent, due to the charge they carry in the transmembrane field[16]. In addition, OCBs is lack of effectiveness when applied before the agonist challenge. In the present experiments, the inhibition of corticosterone on I_{ACh} was voltage-independent, and the result of pretreatment with corticosterone showed that corticosterone had access to the ACh receptor complex even in closed conformation, thereby augmented the inhibition of corticosterone on I_{ACh}. It suggests that the inhibition produced by corticosterone does not depend on the conformation of the ACh receptor channel complex. The results of kinetic analysis of the desensitization process would provide strong support to this idea. The decay observed during the simultaneous application of ACh and corticosterone was fitted well by a double exponential function with time constants of τ_f and τ_s. In fact, τ_s was in correspondence with τ of the control value, and just another faster exponential component (τ_f) was added. The fast exponential function might reflect a mixed process of true desensitization and gradual attainment of corticosterone binding and its consequent inhibition. Binding of corticosterone, probably to the nACh receptor complex, may be a slow process compared with ACh activation kinetics. When the interaction between corticosterone and its binding site reached a steady state, application of ACh did not modify this state. Therefore the conformational change of the ACh receptor complex did not affect the affinity of corticosterone for its binding site. So the decay pattern of the I_{ACh} was almost identical to the control pattern.

Glucocorticoids have been reported to increase the production of cAMP in human lymphocytes[17], and then cAMP-dependent protein kinase was activated, which was demonstrated to be involved in the phosphorylation of nicotinic receptors and the rate of desensitization was facilitated[18]. But we found there was no effect of 8-Br-cAMP on either peak response or the desensitization[11]. Therefore inhibition by corticosterone may not be mediated by cAMP-dependent phosphorylation.

We postulate that inhibition by corticosterone results from a negative heterotopic interaction between the ACh binding site and a specific corticosterone binding site. The most probable mechanism of inhibitory actions of corticosterone may be channel block. The channel may be physically plugged from outside of the membrane by a corticosterone molecule, and its binding may not be affected by the conformational change of the receptor complex. The inhibition by corticosterone may be a decrease in the total number of the nAChR that can be activated. During stress, GC increases acutely which can inhibit I_{ACh}, thus controlling the immediate catecholamine release from the sympathetic cells.

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糖皮质激素对神经元烟碱受体的调制作用

石丽君[1], 刘玲爱, 程晓红, 王春安[2]
(解放军北京军医学院生理教研室, 北京 100071;
[2]第二军医大学生理教研室, 上海 200433, 中国)

关键词 糖皮质激素类; 烟碱受体; 膜片箝技术

目的: 研究糖皮质激素皮质酮对 PC12 细胞烟碱受体的非基因组作用. **方法**: 采用全细胞膜片箝技术记录神经生长因子分化的 PC12 细胞上的乙酰胆碱(ACh)诱发电流(I_{ACh}). **结果**: PC12 细胞上 I_{ACh}是由烟碱受体引起的. 同时给予 ACh (30 μmol/L)和皮质酮(0.1 - 10 μmol/L) 加速 I_{ACh}的衰减且轻微抑制 I_{ACh}峰值. 用相同浓度的皮质酮预处理细胞可加强对 I_{ACh}峰值的抑制率而不影响受体失敏速率. 牛血清白蛋白耦联的皮质酮(0.1 - 10 μmol/L)对 I_{ACh}有类似于皮质酮的快速抑制作用. 此快速抑制作用呈可逆性、浓度依赖性和非电压依赖性. **结论**: 皮质酮可快速抑制 PC12 细胞 I_{ACh}, 且是由非基因组机制介导的. 皮质酮结合于膜外的特异结合位点, 很可能直接作用于神经元烟碱受体, 以一种非竞争方式抑制 I_{ACh}, 从而调控交感神经元的儿茶酚胺释放.

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