通道离子选择性在445，447位置处分别通过阳离子-π轨道作用机理和氧笼机理来实现；(3) CTX和AgTx2与孔区的不同结合方式导致了它们通道亲和力的差异；(4) 孔区内侧静电势主要为负. 结论：构建的模型与从实验结果导出的限制信息是相一致的.

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Kinetic properties of nicotinic receptors in cultured rat sympathetic neurons from superior cervical ganglia[1]

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KEY WORDS nicotine; nicotinic receptors; sympathetic ganglia; pharmacokinetics; binding sites; patch-clamp techniques

AIM: To analyze the kinetic properties of the effect of nicotine on nicotinic acetylcholine receptors (*nAChR*) *in the cultured sympathetic neurons from* neonatal rat superior cervical ganglia (SCG). **METHODS:** The whole-cell recording method of patch-clamp technique was used to record the currents induced by different concentrations of nicotine. The concentration-response of nicotine was fitted with Clark equation. **RESULTS:** Hill coefficient (1.097) was determined by fitting the nicotine responses of neuronal *nAChR* with Clark equation. The theoretical values of nicotine effect, calculated with Clark equation with $H = 1$, were basically identical with the practically recorded currents. **CONCLUSIONS:** Interaction of nicotine and *nAChR* in rat SCG fits a single binding site model.

Analyzing the kinetics of the effect of a drug on the target receptor is very important for elucidating its pharmacological properties and reactive mechanism. The first model to explain interacting process of a receptor and its ligand was put forward by Clark[1], from which a sequential model to describe the concentration dependence of acetylcholine (ACh) action was provided[2,3].

$$nA + R \overset{K}{\rightleftharpoons} AnR \overset{\beta}{\rightleftharpoons} AnR^* \quad (1)$$

In the sequential model 1, a receptor (R) can be activated by ACh (A) by forming an inactive ACh-receptor complex, where AnR and AnR^* are inactive and active agonist-receptor complexes, respectively. And n is the number of ACh molecules activating a single receptor channel. According to the above scheme, the Clark equation could be transformed into the following equation:

$$E = E_{max} \cdot A^H / (K + A^H) \quad (2)$$

In the equation 2, E = response induced by nAChR agonist at a given concentration; E_{max} = the largest response of nAChR; A = agonists; K = equilibrium dissociation constant; H = Hill coefficient, just equating to n in the sequential model 1.

The sequential model is now widely used for the kinetic analysis of acetylcholine-receptor interaction of end-plate currents and single-channel currents of muscle nAChR[4]. There are 2 ligand binding sites on the muscle nAChR and 2 molecules of agonist are required to excite a receptor molecule[5,6] (*ie* $H = 2$). However, in the central nervous system, receptor binding assays have revealed a single class of binding sites in neuronal nAChR[7-9]. Neuronal nAChR display a complicated diversity of the structure and biological functions compared with nAChR in skeletal muscles[10]. It is still uncertain that whether 1 or 2 molecules of an agonist are required to activate 1 molecule of neuronal nAChR in sympathetic

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ganglia. To answer this question, we have used the whole-cell recording method to study the interaction between nicotine and nAChR in SCG neurons.

MATERIALS AND METHODS

Cell culture All the experiments were conducted on dissociated sympathetic neurons derived from superior cervical ganglia (SCG) of neonatal Wistar rats 1 d old[11]. The ganglia were isolated and cut into small pieces which were then incubated in L-15 Leibovitz medium containing 0.25 % trypsin at 36 ℃ for 40 min. The tissue suspensions were spun at 500 × *g* for 2 min. The resulting pellet was gently resuspended in Dulbecco's modified Eagle medium containing 10 % horse serum. Mechanically dissociated neurons were transferred to 35-mm poly-*L*-lysine coated dishes and were maintained at 37 ℃ in 95 % O_2 + 5 % CO_2 environment. Nerve growth factor 50 μL was added to each dish. The experiments were done after the neurons were cultured for 7 – 9 d. In this period, SCG neurons grew well with diameters of 20 – 40 μm and displayed a stable response to nAChR agonists.

Current recording Neurons were voltage-clamped by using whole-cell patch-clamp techniques[12]. When the gigaseal between the tip of the glass microelectrode and the contact membrane of the cultured SCG neuron formed, a swift pulse of suction was applied to the microelectrode interior to break the membrane and establish a whole-cell recording configuration. The membrane potential was held at – 70 mV. All experiments were done at room temperature (20 – 25 ℃) with a Axopatch-1D amplifier. The gathered signals were recorded onto the harddisk of a computer by data acquisition/analysis program pCLAMP 5.5.1 (Axon Instruments).

The patch-pipettes had a resistance of 1 – 3 MΩ and contained: CsCl 140, HEPES 10, egtazic acid 10, and ATP 2 mmol·L^{-1}. The extracellular solution contained: NaCl 140, KCl 5, $CaCl_2$ 3, $MgCl_2$ 1, HEPES 10, glucose 10, and tetrodotoxin 0.001 mmol·L^{-1}.

Application of agonists Nicotine was applied by a puff pipette which was connected to a pressure injector (BH-2, Medical Systems Co). The pipette consisted of 7 microtubes filled with different concentrations of nicotine. The diameter of the microtube was 5 – 10 μm. The distance between the pipette and the neuron was 20 – 30 μm, and 50 – 60 kPa of N_2 pressure was applied. The application time was 1 s and interval was 5 min. Nicotine was purchased from Sigma Chemical Co.

RESULTS

When nicotine was applied to the neuron, it elicited a rapid inward current. The amplitudes (nA) of currents induced by nicotine 10, 20, 40, 80, and 160 μmol·L^{-1} were 0.91 ± 0.08, 1.56 ± 0.14, 2.53 ± 0.27, 3.93 ± 0.46, and 4.57 ± 0.55 (n = 15), respectively (Fig 1). The data were fitted with the Clark equation 2 with different H coefficients.

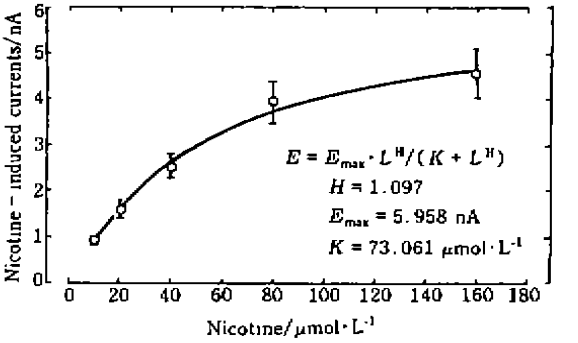

Fig 1. Fitting curve with Clark equation.

When Hill coefficient was regarded as a variable parameter, the obtained H was equal to 1.097, near to one. If Hill coefficient was assumed as 1 or 2, the calculated K values were 61.457 or 667.707 μmol·L^{-1}, respectively. According to Clark's occupation theory, K was the agonist concentration when 50 % receptors became the agonist-occupied receptors or the response of agonist reached up to the half of the maximum efficiency. At $H = 2$, the obtained K value would be much higher (667.707 μmol·L^{-1}) than that of the highest concentration (160 μmol·L^{-1}) of nicotine used in the experiment (Tab 1).

Tab 1. Fitting concentration-response curve of nicotine with Clark equation.

Fitting equations	Hill coefficient	E_{max}/nA	K/μmol·L^{-1}
$E = E_{max} \cdot A^H/(K + A^H)$	1.097	5.958	73.061
$E = E_{max} \cdot A(K + A)$	1	6.513	61.457
$E = E_{max} \cdot A^2/(K + A^2)$	2	4.279	667.707

Obviously, Hill coefficient could not be equal to 2. Whether or not it was really feasible for H = 1? To answer this question, E values were estimated with E_{max} = 6.513 nA and K = 61.457 μmol·L^{-1}

(both were obtained with $H = 1$, Tab 1) at different concentrations of nicotine. The theoretical values were basically identical with the recorded currents ($P > 0.05$, Tab 2).

Tab 2. Comparison between amplitudes of nicotine-induced *currents and calculated values* [$E = E_{max} \cdot A/(K + A)$]. $n = 15$ neurons, $\bar{x} \pm s$. Paired t test: $^aP > 0.05$ *vs* recorded amplitudes.

Nicotine/ $\mu mol \cdot L^{-1}$	Nicotine-induced currents/nA		Differences/ %
	Recorded amplitudes	Calculated values	
10	0.91 ± 0.08	0.91[a]	−0.57
20	1.56 ± 0.14	1.60[a]	−2.64
40	2.53 ± 0.27	2.57[a]	−1.50
80	3.93 ± 0.46	3.68[a]	6.23
160	4.57 ± 0.55	4.71[a]	−3.03

DISCUSSION

Muscle nAChR consists of $(\alpha 1)_2\beta 1\varepsilon\delta$ subunits (adult muscle) and α subunits possess essential elements for the binding of ACh[13]. In neuronal nAChR, two α subunits and three of the β-type subunits associate to form a functional pentameric receptor except for α7 neuronal nAChR[8]. Unlike muscle nAChR, neuronal nAChR displayed a single class of binding sites in different brain regions and spinal cord of rats[7-9].

In sympathetic ganglionic neurons, the expressed genes of nAChR were suggested to be α3, β2 and β4 according to their pharmacological properties[14,15]. The properties of the binding sites of nAChR in sympathetic ganglia were not clear. In this experiment, the responses of neuronal nAChR from rat SCG to different concentration nicotine were fitted with Clark equation and obtained Hill coefficient was near to one ($H = 1.097$). The theoretical values calculated with E_{max} and K (both were obtained by fitting the concentration-response curve with Clark equation with $H = 1$) were basically consistent with the currents evoked by different concentrations of nicotine. The results first demonstrated that nicotine fit an one-site model of receptor-ligand interaction on neuronal nAChR in SCG. This means that a molecule of nAChR in sympathetic ganglia would be activated by a molecule of agonist.

Is there only one of the two α subunits carrying the agonist recognition site in neuronal nAChR structurally? The problem remains unresolved. Perhaps a more likely explanation was that each of the two α subunits would have its own binding site for agonists. But while either of the two binding site was occupied by a molecule of agonist, the receptor would be activated and the ion channel opened[10]. So, the relationship of molecule equivalent between neuronal nAChR and its agonist would display a ratio of one to one ($H = 1$). Of course, the further experiments are needed to elucidate the original situation of interaction between neuronal nAChR and its agonists.

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培养的大鼠颈上神经节交感神经元烟碱受体的动力学特性[1]

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R969.1

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关键词 烟碱; 烟碱受体; 交感神经节; 药物动力学; 结合位点; 膜片箝技术

目的: 研究培养的新生大鼠颈上神经节交感神经元烟碱受体的动力学特性. 方法: 膜片箝技术的全细胞记录方法, 记录不同浓度烟碱诱发的电流, 使用 Clark 方程对烟碱作用的量效曲线进行拟合. 结果: 10, 20, 40, 80 和 160 μmol·L^{-1}烟碱诱发电流的幅度分别为: 0.91 ± 0.08, 1.56 ± 0.14, 2.53 ± 0.27, 3.93 ± 0.46 和 4.57 ± 0.55 nA (n = 15), 经 Clark 方程拟合, 得到 H = 1.097, E_{max} = 5.958 nA, K = 73.061 μmol·L^{-1}, 将 H = 1 时拟合得到的 E_{max}(6.513 nA)和 K 值(61.457 μmol·L^{-1})代入 Clark 方程, 所计算出的理论值与相应浓度烟碱诱发电流的实测值基本相符. 结论: 烟碱与交感神经元烟碱受体作用的动力学特性符合一个作用位点的反应模型.

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Electrophysiologic effect of enalapril on guinea pig papillary muscles *in vitro*

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KEY WORDS enalapril; action potentials; papillary muscles; ouabain; electrophysiology

AIM: To study the direct effect of enalapril on cellular electrophysiology of myocardium.
METHODS: Conventional microelectrodes technique was used to record the action potentials (AP) of guinea pig papillary muscles. **RESULTS**: Enalapril caused an increase of the AP amplitude (APA) and the resting potential (RP) in a concentration-dependent manner without any significant change of AP duration, V_{max} and overshoot of AP. Superfusion of ouabain 0.5 μmol·L^{-1} reduced APA and RP, induced stable delayed after-depolarizations (DAD) at different basic cycle lengths (BCL) in a frequency-dependent manner. At BCL 200 ms, the amplitude of DAD was large enough to induce nonsustained triggered activity (TA). In additional presence of enalapril 10 μmol·L^{-1}, the DAD amplitude at 500, 400, 300, and 200 ms were decreased from 5.3 ± 2.3, 5.9 ± 2.8, 7.4 ± 2.1, and 8.9 ± 1.3 to 2.6 ± 0.7, 3.1 ± 1.0, 3.7 ± 1.5, and 5.3 ± 1.1 (mV) respectively, all $P < 0.01$. The compensation intervals were increased in a similar frequency-dependent manner. The number of TA induced at BCL 200 ms was decreased from 3.6 ± 0.7 to 0.8 ± 0.2 ($P < 0.05$). **CONCLUSION**: Enalapril directly inhibits DAD and TA induced by ouabain through increasing RP and APA, which may contribute to its anti-arrhythmic effect.

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