脑后部牛磺酸的释放增加；相反，通过失血使血容量减少或静脉注射硝普钠引起血压下降时，下丘脑后部牛磺酸的释放减少.

**结论**：下丘脑后部牛磺酸对血压的中枢调节起着很重要的作用.

**关键词** 牛磺酸；下丘脑后部；灌注法；血压；血容量；去甲肾上腺素；硝普钠

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# Relationship between muscarinic receptor subtypes and cyclic nucleotides in pons-medulla oblongata[1]

GE Xiao-Qun, HU Gang, YAO Bing, XU Peng-Cheng, BIAN Chun-Fu
(*Department of Pharmacology, Xuzhou Medical College, Xuzhou 221002, China*)

**AIM**: To study the relationship between muscarinic receptor (M-R) subtypes and cyclic nucleotides in pons-medulla oblongata (MeOb). **METHODS**: The contents of cGMP and cAMP in Sprague-Dawley rat pons-MeOb, cerebellum and cerebral cortex were assayed by radioimmunoassay and competitive protein-binding assay, respectively, after ip injections of drugs. Control rats were given ip normal saline. **RESULTS**: $M_1$-R agonist pilocarpine (6, 15 mg $kg^{-1}$, ip) increased the content of cGMP in the pons-MeOb and cerebral cortex, but did not bring about any noticeable change in the cAMP content. The increase of cGMP was antagonized by ip pirenzepine or scopolamine. On the other hand, ip $M_2$-R agonist 6β-acetoxy nortropane (6β-AN) 25 μg $kg^{-1}$ reduced not only cAMP contents in the pons-MeOb and cerebellum but also cGMP contents in the pons-MeOb and cerebral cortex, while 6β-AN 12 μg $kg^{-1}$ only lowered cAMP content. The decreases of cGMP and cAMP induced by 6β-AN were antagonized by ip AF-DX 116 or atropine, respectively. **CONCLUSION**: Stimulation of $M_1$-R causes the increase of cGMP and that of $M_2$-R induces the decreases of both cGMP and cAMP in the pons-MeOb.

**KEY WORDS** cyclic GMP; cyclic AMP; muscarinic receptors; pilocarpine; nortropanes; pirenzepine; scopolamine; atropine

Pons and medulla oblongata (MeOb) play an important role in regulation of respiration. We previously found that there were $M_1$ muscarinic receptor ($M_1$-R, 30 %— 40 %) and $M_2$-R (60 %— 70 %) subtypes in pons-MeOb[1], and that the excitation of $M_1$-R stimulated respiration and excitation of $M_2$-R inhibited respiration[1,2]. However, it is not known why the effects of $M_1$ and $M_2$ receptors on respiration are so distinct. Although $M_1$ and $M_2$ receptors were separately coupled to the elevation of cGMP level and the inhibition of cAMP formation *in vitro*[3,4], the relationship between M-R subtypes and the two cyclic nucleotides in pons-MeOb remains to be defined. In the present study, the contents of cGMP and cAMP in rat pons-MeOb, cerebral cortex, and cerebellum were assayed after ip injections of $M_1$-R agonist pilocarpine[5,6] and

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antagonist pirenzepine or scopolamine, and $M_2$-R agonist 6β-acetoxy nortropane (6β-AN)[6] and antagonist AF-DX 116[7] or atropine.

## MATERIALS AND METHODS

**Drugs** Pirenzepine (Pir) was synthesized and presented by Chongqing Pharmaceutical Research Institute, Sichuan. AF-DX 116 (11-[[2-[(diethylamino) methyl]-1-piperidinyl] acetyl]-5, 11-dihydro-6H-pyrido [2, 3-b] [1, 4] benzodiazepine-6-one) was purchased from Karl Thomae GmbH Chemisch-Pharmazeutiche Fabrik, Germany. Pilocarpine (Pil) was Sigma product. 6β-AN was synthesized by Department of Chemistry, Shanghai Second Medical University, Shanghai. Scopolamine (Sco) was Merck product. Atropine (Atr) was made by Chengdu First Pharmaceutic Factory, Sichuan. The reagent kits for cGMP and cAMP analyses were purchased from Chinese Academy of the Atomic Energy and Institute of Basic Medicine, Chinese Academy of Medical Sciences, Beijing, respectively.

**Determination of cGMP and cAMP** Sprague-Dawley rats of 3—3.5 months ($n=60$, 224±$s$ 22 g) were decapitated 30 min after ip injection of drugs. Control rats ($n=6$) were given ip normal saline. The cerebral cortex, cerebellum, and pons-MeOb were frozen in liquid nitrogen. The contents of cGMP and cAMP were determined by radioimmunoassay and competitive protein-binding assay, respectively according to the instruction attached to the reagent kits.

**Statistics** The data were analyzed by $t$ test.

## RESULTS

Pil 6 and 15 mg $kg^{-1}$ ip induced the dose-dependent increases of cGMP contents in pons-MeOb and cerebral cortex, while the contents of cAMP in pons-MeOb and cerebellum did not change significantly. When Pil (15 mg $kg^{-1}$, ip) was given in combination with Pir (20 mg $kg^{-1}$, ip) or Sco (20 mg $kg^{-1}$, ip), its cGMP-increasing action was markedly weakened (Tab 1).

When 6β-AN 12 and 25 μg $kg^{-1}$ was ip injected, the contents of cAMP in pons-MeOb and cerebellum were decreased in a dose-dependent manner. AF-DX 116 (3 mg $kg^{-1}$, ip) and Atr (20 mg $kg^{-1}$, ip) antagonized this action (Tab 1).

The increase of cGMP content following ip injection of 6β-AN 12 μg $kg^{-1}$ was not much, nor was it antagonized by AF-DX 116. However, 6β-AN 25 μg $kg^{-1}$ obviously lowered the content of cGMP in pons-MeOb and

**Tab 1. Effects of pilocarpine (Pil), pirenzepine (Pir), scopolamine (Sco), 6β-AN, AF-DX 116, and atropine (Atr) ip on cGMP and cAMP in rat brain.** $n=6$ rats, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* saline. $^dP>0.05$, $^eP<0.01$ *vs* 6β-AN. $^hP<0.05$, $^iP<0.01$ *vs* PH 15 mg $kg^{-1}$.

| Group | cGMP/pmol $g^{-1}$ | | cAMP/nmol $g^{-1}$ | |
|---|---|---|---|---|
| | Pons-MeOb | Cerebral cortex | Pons-MeOb | Cerebellum |
| Saline | 119±27 | 135±20 | 2.80±0.35 | 2.27±0.37 |
| Pil 6 mg $kg^{-1}$ | 157±32[b] | 235±48[c] | | |
| 15 mg $kg^{-1}$ | 204±40[c] | 296±49[c] | 2.60±0.20[a] | 2.30±0.26[a] |
| Pil 15 mg $kg^{-1}$+Pir 20 mg $kg^{-1}$ | 134±32[i] | 196±43[i] | | |
| Pil 15 mg $kg^{-1}$+Scop 20 mg $kg^{-1}$ | 156±19[h] | 171±32[i] | | |
| 6β-AN 12 μg $kg^{-1}$ | 138±26[e] | 152±36[e] | 1.02±0.25[c] | 0.66±0.22[c] |
| 25 μg $kg^{-1}$ | 70±20[c] | 92±36[c] | 0.50±0.13[c] | 0.23±0.09[c] |
| 6β-AN 12 μg $kg^{-1}$+AF-DX 116 3 mg $kg^{-1}$ | 142±26[d] | 150±23[d] | 2.21±0.45[f] | 1.86±0.46[f] |
| 6β-AN 25 μg $kg^{-1}$+AF-DX 116 3 mg $kg^{-1}$ | 129±26[f] | 180±31[f] | 1.29±0.23[f] | 0.93±0.26[f] |
| 6β-AN 25 μg $kg^{-1}$+Atr 20 mg $kg^{-1}$ | 156±23[i] | 180±25[i] | 1.60±0.33[i] | 1.16±0.37[i] |

in cerebral cortex. And this action was completely antagonized by ip AF-DX 116 3 mg $kg^{-1}$ as well as by ip Atr 20 mg $kg^{-1}$ (Tab 1).

## DISCUSSION

$M_1$ and $M_2$ receptors mediated cGMP formation and cAMP inhibition, respectively, in N1E-115 cells[3] and dissociated cerebral cortex[4]. Therefore, in the present study, the cerebral cortex that contains rich $M_1$-R[8,9] was used for determining cGMP, while the cerebellum mainly containing $M_2$-R[8,10], for cAMP. The results obtained supported the above stated conclusion. Since rat pons-MeOb contains $M_1$ and $M_2$ receptors and the drugs we used can pass through blood brain barrier in ip injection condition, the changes of cyclic nucleotides in pons-MeOb should be mainly attributed to the direct action of drugs on the tissues. The present results indicate that the increase of cGMP induced by Pil results from stimulation of $M_1$-R in pons-MeOb, as the effect was antagonized by Pir or Sco. So far, there has been no report about the effect of 6β-AN on cyclic nucleotides, especialy its cGMP-decreasing action, which was found for the first time. It is obvious that the decrease of cGMP is not related to $M_1$-R, for stimulating $M_1$-R leads to the increase of cGMP. Our study shows that 6β-AN-induced decreases of cGMP and cAMP are antagonized by AF-DX 116 or Atr in pons-MeOb, indicating that the effects are related to stimulation of $M_2$-R. From above, we conclude that stimulation of $M_1$-R causes the increase of cGMP and that stimulation of $M_2$-R induces the decreases of both cGMP and cAMP in rat pons-MeOb.

At present, many studies on biochemical mechanism of M-R subtypes have been reported[3,4,11-13]. But the relationship between biochemical changes and pharmacological effects has not been clear. Considering our previous study[1], we put forward a hypothesis that respiratory excitation caused by stimulating $M_1$-R and respiratory inhibition caused by stimulating $M_2$-R might be separately related to the increase of cGMP and the decrease of cGMP.

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## 脑桥-延髓中毒蕈碱受体亚型与环核苷酸的关系

葛晓群，胡 刚，姚 兵，计鹏程，卞春甫
(徐州医学院药理教研室，徐州221002，中国)

**目的**：研究脑桥-延髓中 M 受体亚型与环核苷酸的关系. **方法**：大鼠 ip 药物，脑桥-延髓等组织中 cGMP 和 cAMP 含量分别用放射免疫法和竞争性蛋白结合法测定，对照组 ip 生理盐水. **结果**：ip 匹鲁卡品，脑桥-延髓中 cGMP 含量增加，cAMP 变化不明显，ip 哌仑西平或东莨菪碱可拮抗之. ip 6β-乙酰氧基去甲托烷 12 μg $kg^{-1}$，脑桥-延髓中 cAMP 含量减少，而 25 μg $kg^{-1}$使 cAMP 和 cGMP 均减少，ip AF-DX 116或阿托品可拮抗之. **结论**：激动脑桥-延髓中 $M_1$受体使 cGMP 增加，激动 $M_2$受体使 cGMP 和 cAMP 减少.

**关键词** 鸟苷环一磷酸；腺苷环一磷酸；毒蕈碱受体；匹鲁卡品；去甲托烷；哌仑西平；东莨菪碱；阿托品

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Please contact Prof KURAHASHI Kazuyoshi
Pharmacology Division
Radioisotope Research Center
Kyoto University
Sakyo-ku, Kyoto 606
JAPAN
Phone: 81-75-753-7514. Fax: 81-75-753-7504.