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Effects of M_1 and M_2 receptor agonists and blockers on dog respiration[1]

YAO Bing, GE Xiao-Qun, ZHENG Jia-Lin, QIN Wei, BIAN Chun-Fu
(*Department of Pharmacology, Xuzhou Medical College, Xuzhou 221002, China*)

KEY WORDS muscarinic receptors; pilocarpine; nortropanes; scopolamine; pirenzepine; atropine; respiration

AIM: To study the effects of M_1 and M_2 receptor agonists and blockers on dog respiration. **METHODS**: Using thoracic respiratory transducer and RM-86 multipurpose polygraph to determine respiratory rate (RR), tidal volume (TV), and minute ventilation volume (MVV), and DH-100G blood gas analysis instrument to analyze pO_2, pCO_2 and pH. **RESULTS**: Pilocarpine (Pil, an M_1-R subtype agonist) 0.5, 1, and 2 mg·kg^{-1} iv caused increases in RR, MVV, and pO_2, and a decrease in pCO_2. The excitatory effects of Pil were antagonized by pretreatment with pirenzepine (Pir, 3 mg·kg^{-1}, iv) and scopolamine (Sco, 2 mg·kg^{-1}, iv). The iv injections of a novel M_2-R subtype agonist, 6β-acetoxy nortropane (6β-AN) 2, 5, and 20 μg·kg^{-1} caused decreases in RR, MVV, and pO_2 and an increase of pCO_2. The actions of 6β-AN were antagonized by iv pretreatment with AF-DX116 [11-2 [[2-[(diethylamino) methyl]-1-piperidinyl] acetyl]-5, 11-dihydro-6H[2, 3-b][1, 4]benzodiazepine-6-one, 0.5 mg·kg^{-1}] and atropine (Atr, 2 mg·kg^{-1}). Similar results were obtained when smaller doses of Pil (0.2, 0.4, and 0.8 mg·kg^{-1}) and 6β-AN (0.25, 0.5, and 1 μg·kg^{-1}) were injected into the vertebral artery. Pir and Sco also antagonized the excitatory effects of Pil, and AF-DX116 and Atr antagonized the inhibitory effects of 6β-AN on respiration. **CONCLUSION**: Stimulating M_1-R of the respiratory center caused excitation of the respiration while stimulating the M_2-R subtype caused inhibition of the respiration.

There are M_1 and M_2 cholinergic receptors in the respiratory center of rats, and in rabbits, Pilocarpine (Pil, an M_1-R agonist) causes an excitatory effect on respiration, while 6β-acetoxy nortropane (6β-AN, an M_2-R agonist) causes an inhibitory effect not only in rabbits but in dogs[1-4]. In the present study, experiments on respiratory function were used to determine the effects of Pil, 6β-AN, pirenzepine (Pir), and scopolamine (Sco) (M_1-R antagonists) and 11-2 [[2-[(diethylamino)methyl]-1-piperidinyl]acetyl]-5, 11-dihydro-6H[2, 3-b][1, 4]benzodiazepine-6-one (AF-DX116) and Atropine (Atr) (M_2-R antagonists) by various modes of administration in dogs and to study the relationships between the central cholinergic system and respiration.

MATERIALS AND METHODS

Mongrel dogs of either sex, weighing 12.3 ± s 2.1 kg (n = 83) were used. Forty of them were anesthetized with pentobarbital 20 mg·kg^{-1} iv to cannulate the vertebra artery for drug injection (ia). The respiratory rate (RR), tidal volume (TV) and minute ventilation volume (MVV) were determined by thoracic respiratory transducer and RM-86 multipurpose polygraph[5]. Arterial blood was analyzed for pO_2, pCO_2, and pH with DH-100G blood gas analysis instrument. In antagonistic test, blockers were injected iv or ia 10 min before agonists.

Drugs Pil (Sigma Co), 6β-AN (Department of Chemistry, Shanghai Second Medical University), Pir (Chongqing Institute of Materia Medica), AF-DX116 (Karl Thomae GmbH Chemisch-Pharmazeutiche Fabrik, Germany), Sco hydrobromide and Atr sulfate (Chengdu First Pharmaceutical Plant).

RESULTS

1 Effects of Pil iv or ia on dog respiration

Pil 0.5, 1, 2 mg·kg^{-1} iv or 0.4, 0.8 mg·kg^{-1} ia caused increases in RR and MVV and a decrease in pCO_2 in a dose-dependent manner. TV and pO_2 did not show remarkable increase. After Pil 2 mg·kg^{-1} iv the maximal increases of RR and MVV occurred at 10 min. The excitatory effects of

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respiration lasted >60 min. These effects of Pil were antagonized by pretreatment of Pir 3 mg·kg^{-1} or Sco 2 mg·kg^{-1} iv. The antagonistic effects of Pir or Sco began 10 min after iv and lasted >1 h. In Pil 0.8 mg·kg^{-1} ia group, the excitatory effect on respiration started 10 min after ia and persisted for >1 h. Its maximal effect mainfested at 30 min. The excitatory effect of Pil 0.8 mg·kg^{-1} was antagonized when Pir 2 mg·kg^{-1} or Sco 1 mg·kg^{-1} was given jointly, and increases of RR and MVV were more less than those when Pil was used alone (Fig 1, Tab 1).

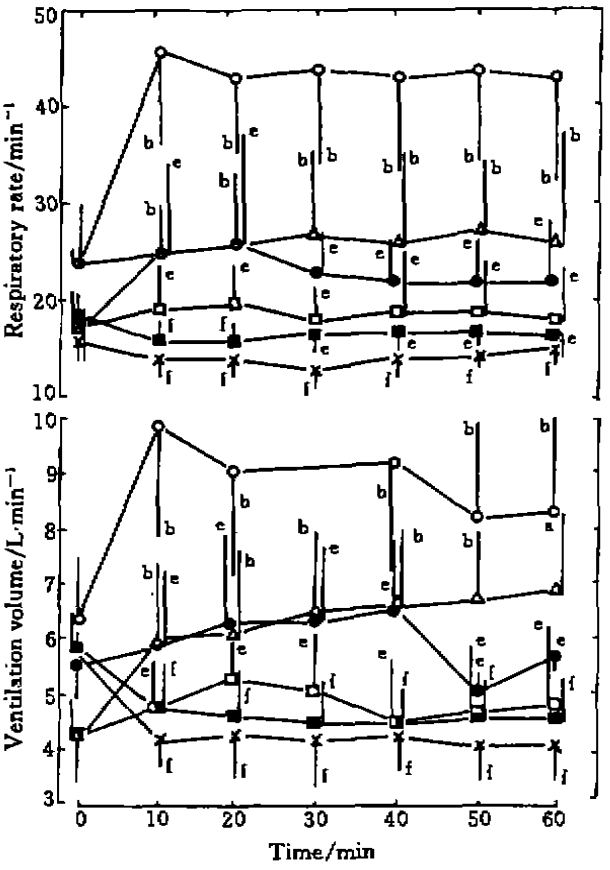

Fig 1. Effects of pilocarpine (Pil) alone and in combination with scopolamine (Sco) or pirezepine (Pir) on respiratory rate in dogs. (○) **Pil 2 mg·kg^{-1} iv,** (●) **Sco 2 mg·kg^{-1} + Pil 2 mg·kg^{-1} iv.** (×) **Pir 3 mg·kg^{-1} + Pil 2 mg·kg^{-1} iv,** (△) **Pil 0.8 mg·kg^{-1} ia,** (□) **Sco 1 mg·kg^{-1} + Pil 0.8 mg·kg^{-1} ia,** (■) **Pir 2 mg·kg^{-1} + Pil 0.8 mg·kg^{-1} ia.** $^{a}P>0.05$, $^{b}P<0.05$, $^{c}P<0.01$ *vs* **0 min;** $^{d}P>0.05$, $^{e}P<0.05$, $^{f}P<0.01$ *vs* **Pil alone.**

2 Effects of 6β-AN iv or ia on dog respiration 6β-AN 2, 5, 20 μg·kg^{-1} iv caused dose-dependent decreases of RR, MVV, and pO_2, and increase of pCO_2. The inhibitory effect began at 10 min and became most marked at 30 min. These actions, except pO_2, were antagonized by AF-DX116 0.5 mg·kg^{-1} or Atr 2 mg·kg^{-1}. 6β-AN (0.2, 0.5, 1 μg·kg^{-1}) ia induced similar results as 6β-AN (2, 5, 20 μg·kg^{-1}) iv. The RR and MVV were decreased in a dose-dependent manner. The pO_2 and pCO_2 did not change markedly. In 6β-AN 1 μg·kg^{-1} group, the inhibitory effect on respiration was most marked at 10 min and lasted >1 h. These inhibitory effects were reversed by addition of AF-DX116 0.2 mg·kg^{-1} or Atr 0.5 mg·kg^{-1} ia. Consequently, increases in RR and MVV and decrease in pCO_2 were noted (Fig 2, Tab 1).

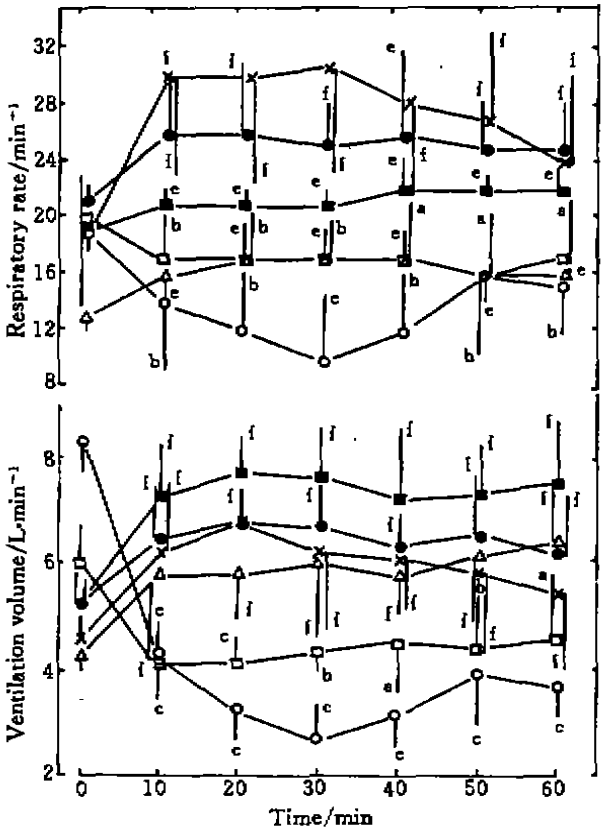

Fig 2. Effects of 6β-AN alone and in combination with AF-DX116 or atropine (Atr) on respiratory rate in dogs. (○) **6-AN 20 μg·kg^{-1} iv,** (●) **AF-DX116 0.5 mg·kg^{-1} + 6β-AN 20 μg·kg^{-1} iv.** (×) **Atr 2 mg·kg^{-1} + 6β-AN 20 μg·kg^{-1} iv,** (□) **6β-AN 1.0 μg·kg^{-1} ia,** (■) **AF-DX116 0.2 mg·kg^{-1} + 6β-AN 1.0 μg·kg^{-1} ia,** (△) **Atr 0.5 mg·kg^{-1} + 6β-AN 1.0 μg·kg^{-1} ia.** $^{a}P>0.05$, $^{b}P<0.05$, $^{c}P<0.01$ *vs* **0 min;** $^{d}P>0.05$, $^{e}P<0.05$, $^{f}P<0.01$ *vs* **6β-AN alone.**

Tab 1. Arterial pO_2 and pCO_2 after pilocarpine (Pil), and 6β-AN, pirenzepin (Pir), scopolamine (Sco), AF-DX116, and atropine (Atr). $n = 4$ or 5, $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* before.

Drugs				Dogs	pO_2/kPa		pCO_2/kPa	
					Before	After	Before	After
Pil	Pri	Sco						
	($mg \cdot kg^{-1}$)							
0.2			ia	5	10.9 ± 0.4	11.5 ± 0.7[a]	3.9 ± 2.0	3.5 ± 1.8[a]
0.4			ia	5	10.9 ± 0.4	11.3 ± 0.9[a]	3.9 ± 2.0	3.3 ± 1.9[a]
0.8			ia	5	10.9 ± 0.4	11.6 ± 1.1[a]	3.9 ± 2.0	3.0 ± 1.8[b]
0.8	2.0		ia	4	10.5 ± 0.9	11.5 ± 0.4[b]	5.1 ± 0.6	4.4 ± 0.4[a]
0.8		1.0	ia	4	10.5 ± 0.8	10.9 ± 1.1[b]	5.4 ± 0.5	5.2 ± 0.9[a]
0.5			iv	5	12.0 ± 0.8	11.7 ± 0.7[a]	4.3 ± 0.7	4.0 ± 0.7[a]
1.0			iv	5	12.0 ± 0.8	11.2 ± 1.7[a]	4.3 ± 0.7	3.5 ± 0.6[b]
2.0			iv	5	12.0 ± 0.8	10.3 ± 2.1[a]	4.3 ± 0.7	3.5 ± 0.5[b]
2.0	3.0		iv	4	8.7 ± 0.3	9.9 ± 0.8[a]	4.7 ± 0.4	3.9 ± 0.2[a]
2.0		2.0	iv	5	12.0 ± 0.8	12.0 ± 0.4[a]	3.0 ± 0.5	2.8 ± 0.4[a]
6β-AN	AF-DX116	Atr						
($\mu g \cdot kg^{-1}$)	($mg \cdot kg^{-1}$)							
0.2			ia	4	9.2 ± 0.7	9.2 ± 0.5[a]	5.4 ± 1.7	4.0 ± 0.7[a]
0.5			ia	4	9.2 ± 0.7	8.9 ± 1.9[a]	5.4 ± 1.7	5.7 ± 2.7[a]
1.0			ia	4	9.2 ± 0.7	9.3 ± 1.8[a]	5.4 ± 1.7	5.3 ± 2.8[a]
1.0	0.2		ia	4	8.8 ± 1.6	10.8 ± 0.9[b]	5.6 ± 1.4	4.4 ± 0.8[b]
1.0		0.5	ia	4	10.4 ± 0.5	9.7 ± 1.3[a]	6.1 ± 1.4	5.1 ± 1.3[b]
2.0			iv	5	13.6 ± 1.9	12.8 ± 1.5[b]	4.1 ± 0.6	4.6 ± 0.7[b]
5.0			iv	5	13.9 ± 2.0	12.5 ± 0.9[b]	4.0 ± 0.5	4.9 ± 0.5[b]
20.0			iv	5	13.2 ± 1.1	11.9 ± 0.3[b]	4.0 ± 0.4	5.3 ± 0.4[c]
20.0	0.5		iv	4	10.4 ± 0.4	11.9 ± 1.7[a]	4.9 ± 0.3	3.7 ± 0.4[b]
20.0		2.0	iv	5	11.1 ± 0.4	10.9 ± 1.9[a]	4.4 ± 0.6	3.5 ± 0.4[b]

3 Cross antagonistic test When AF-DX116 (0.5 $mg \cdot kg^{-1}$ iv or 0.2 $mg \cdot kg^{-1}$ ia) and Pil (2 $mg \cdot kg^{-1}$ iv or 0.4 $mg \cdot kg^{-1}$ ia) were coadministered to dogs the RR increased from 22 ± 3 and 19 ± 5 time $\cdot min^{-1}$ to 46 ± 3 and 27 ± 11 time $\cdot min^{-1}$, and MVV from 3.2 ± 0.6 and 4.2 ± 0.2 $L \cdot min^{-1}$ to 5.7 ± 0.9 and 7.3 ± 1.6 $L \cdot min^{-1}$. pCO_2 decreased too. These effects were more intensive than Pil alone and lasted > 1 h. On the other hand, the inhibitory effects of 6β-AN were not antagonized by Pir. The TV was decreases from 368 ± 86 and 313 ± 111 mL to 224 ± 56 and 204 ± 58 mL, and MVV from 6.8 ± 1.4 and 7.0 ± 0.5 $L \cdot min^{-1}$ to 5.5 ± 1.2 and 5.6 ± 0.4 $L \cdot min^{-1}$, and pCO_2 increase by Pir (3 $mg \cdot kg^{-1}$ iv or 2 $mg \cdot kg^{-1}$ ia) and 6β-AN (20 $\mu g \cdot kg^{-1}$ iv or 1 $\mu g \cdot kg^{-1}$ ia) respectively. The inhibitory effects lasted > 1 h too.

DISCUSSION

In the present experiment, Pil iv caused respiratory excitation and the action could be antagonized by Pir. On the other hand, 6β-AN caused inhibitory effects on dog's respiration. These effects could be antagonized by AF-DX116. However AF-DX116 iv could not antagonized the excitatory action of Pil and Pir could not antagonized the inhibitory effects of 6β-AN too. While Pil and 6β-AN are selective agonist of M_1 and M_2 cholinergic receptor respectively[6,7], and Pir and AF-DX116 are blocking agents of M_1 and M_2-R respectively. Thus, it is evident that Pil stimulates respiration by activating the M_1-R subtype, while the inhibitory effects of 6β-AN are brought about by acting upon the M_2-R subtype. As the results showed Sco resembled Pir and antagonized respiratory excitation of Pil; Atr resembeled AF-DX116 and could antagonize respiratory inhibition of 6β-AN. It showed that Sco acted mainly on M_1-R and Atr on M_2-R respectively in this test.

Similar results were achieved when small doses

of the drugs were ia. Pil (ia) caused respiratory excitation, but the effect appeared later and was weaker than that by iv route. The difference was probably related to the fact that the dog was under pentobarbital anesthesia and the dose of Pil was relatively small. However, the excitatory action of Pil ia could also be antagonized by M_1-R blocking agents Pir and Sco but not by M_2-R blocking agent AF-DX116. 6β-AN ia inhibited respiration, with the maximum effect appearing earlier than that by iv route, and could be antagonized by AF-DX116 and Atr but not by M_1-R blocking agent Pir. These results suggested that Pil stimulate respiration mainly by activating the central M_1-R subtype, while the inhibitory effects of 6β-AN be mainly brought about by acting upon the central M_2-R subtype.

Recently, our receptor binding assays with [^{3}H] quinuclidinyl benzilate and [^{3}H] pirenzepine demonstrated the presence of M_1 and M_2 subtypes of M cholinergic receptors in the pons and medulla of rats. The M_1 cholinergic receptor was found to account for approximately 30 % - 40 % of the total muscarinic receptors, and the M_2 accounted for about 60 % - 70 %[1]. Observation of efferent phrenic discharges in rabbits showed that Pir and Sco inhibited the respiratory center, while AF-DX116 and Atr excited it[8]. These results also supported the present observations, i e the activation of M_1-R subtype of respiratory center caused excitatory effects while that of M_2-R subtype brought about inhibitory effects.

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M_1、M_2 受体激动剂和阻滞剂对犬呼吸的影响

姚 兵, 葛晓群, 郑加林, 秦 伟, 卞春甫
(徐州医学院药理教研室, 徐州 221002, 中国)

关键词 毒蕈碱受体; 匹鲁卡品; 去甲托烷类; 东莨菪碱; 哌仑西平; 阿托品; 呼吸

目的: 研究 M_1 和 M_2 受体激动剂和阻滞剂对犬呼吸的影响. **方法**: 用 RM-86 多导生理记录仪通过胸带式呼吸换能器测定 RR, TV 和 MVV, 并取动脉血测 pO_2, pCO_2 和 pH. **结果**: 发现 M_1-R 激动剂 Pil 和 M_2-R 激动剂 6β-AN iv 或椎动脉给药分别产生呼吸兴奋和抑制, RR, MVV 增高或降低($P < 0.05$), 血气亦出现相应变化. M_1-R 阻滞剂 Pir, Sco 和 M_2-R 阻滞剂 AF-DX116, Atr 分别拮抗甚至翻转 Pil 的呼吸兴奋和 6β-AN 的呼吸抑制作用. **结论**: 激动呼吸中枢 M_1-R 呼吸兴奋, 激动呼吸中枢 M_2-R 呼吸抑制, 阻断之则作用相反.

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