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ACTIVATION OF CENTRAL MUSCARINIC RECEPTORS CAUSES RESPIRATORY STIMULATION IN CONSCIOUS ANIMALS

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1 Oxotremorine (10 μg/kg) injected intravenously into conscious rabbits pretreated with atropine-methyl-nitrate (ATMN, 0.5 mg/kg) caused significant increases in respiration rate from 94 to 131 per min, and in Pa_{O_2} from 13.8 to 15.4 kPa, and a decrease in Pa_{CO_2} from 3.30 to 2.09 kPa within 15 min. Blood pH fell from 7.44 to 7.16.

2 Blood pressure increased by 11.6%, 5 min after oxotremorine injection.

3 The acidosis was shown to be due to an increase in blood lactic acid from 41 to 132 mg/100 ml.

4 Pretreatment with propranolol (5 mg/kg, s.c.) prevented the lactic acidosis and fall in pH but did not alter the respiratory stimulation induced by oxotremorine.

5 It is suggested that the lactic acidosis induced by oxotremorine results from stimulation of β-adrenoceptors in skeletal muscle by catecholamines released from the adrenal medulla and sympathetic nerves.

6 Since all the above effects of oxotremorine are antagonized by hyoscine (5 mg/kg) but not by ATMN (0.5 mg/kg), it is concluded that oxotremorine can stimulate respiration by a direct action on muscarinic receptors in the central nervous system.

Introduction

Several studies have been carried out to determine whether cholinergic neurones are involved in the central nervous control of respiration (Lambersten, 1966; Metz, 1966). For this purpose acetylcholine, physostigmine, carbachol and nicotine have each been applied to the medulla oblongata of cats (Comroe 1943; Miller, 1949; Dev & Loeschke, 1979a,b) or infused into the carotid artery of dogs (Gesell & Hansen, 1943).

These studies were of necessity carried out in anaesthetized or decerebrate animals. All the anaesthetics used depress central transmission and pentobarbitone, in particular, has been shown to antagonize specifically the excitation of brain stem neurones by exogenously applied acetylcholine (Bradley & Dray, 1973). This may be why it was necessary to use such high concentrations of these drugs in the perfusion fluid (50–300 μg/ml) to elicit transient stimulation of respiration in the experiments cited above.

It is therefore by no means certain that the drugs acted solely at their site of application in the medulla and did not leak out into the circulation to stimulate peripheral chemoreceptors. This obviously occurred when nicotine was applied to the ventral surface of the medulla because the stimulation of respiration it caused was blocked by intravenously administered hexamethonium, (Dev & Loeschcke 1979b) which does not penetrate the blood brain barrier. This conclusion is supported by an earlier finding that the stimulant effect of nicotine injected into the IVth ventricle did not occur after denervation of the peripheral chemoreceptors (Comroe, 1943).

The respiratory stimulant effect of acetylcholine was not reduced by carotid body denervation (Comroe, 1943) but was antagonized by local application of atropine to the medulla (Dev & Loeschcke, 1979a,b). This suggested that muscarinic receptors in the medulla may mediate the respiratory effect of cholinergic stimulation.

In order to explore in more detail the involvement of central muscarinic receptor activation on respiration, while conforming as far as possible to physiological conditions, we used conscious animals in the present study.

Changes in respiratory activity were monitored by measuring arterial blood gases and pH in response to intravenous injection of oxotremorine. This agent is a specific muscarinic agonist which readily penetrates the central nervous system (Cho, Haslett & Jenden, 1962; George, Haslett & Jenden, 1962). Peripheral muscarinic receptors were blocked by pretreatment with atropine methyl nitrate (ATMN). Under these conditions we were able to demonstrate prolonged respiratory stimulation with doses of oxotremorine as low as 1–10 μg/kg.

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Methods

Male rabbits (mixed strain) weighing 2–2.5 kg were trained to sit quietly in a restraining hammock. One central ear artery was cannulated with a transcutaneous catheter (Quik. Cath. No. 20, Travenol Labs., Ireland, Ltd.) which was filled with sterile saline (0.9% w/v NaCl solution) containing 25 units/ml heparin. The catheter was attached to a pressure transducer by means of a 3-way stopcock, and blood pressure and heart rate recorded on a Brush Gould recorder. Drugs were administered through a butterfly needle (No. 20) placed in the marginal ear vein.

Rectal temperature was monitored on a telethermometer (Yellow Springs Instruments) with the aid of a thermistor probe inserted into the rectum. Respiration rate was counted visually. This was found to be reproducible for ± 10% in rates up to 160 min. Arterial blood samples were taken through the catheter for the measurement of Pa_{CO_2}, Pa_{O_2} and pH on a Corning automatic blood gas analyzer, after correction for the appropriate body temperature.

Rabbits were allowed to rest for at least 1 h under quiet conditions after cannulation, before control readings were taken. Drugs were not administered until consistent values for blood gas tensions and pH had been obtained.

Eight rabbits were injected intravenously with ATMN (0.5 mg/kg) to block peripheral muscarinic receptors and a further blood sample taken 15 min later for blood gas analysis. Oxotremorine (10 μg/kg) was then injected intravenously and its effect was measured on blood pressure and heart rate 2, 5, 10, 15 and 30 min later, and on respiration rate, blood gases and pH after 5, 15, 30 and 60 min. Lactic acid was also measured at these latter times in 0.5 ml samples of blood by the method of Matthews & Sterling (1977), adapted for use on a Union Carbide Centrifichem 400 analyzer.

Eighteen other rabbits were pretreated with propranolol (5 mg/kg s.c.), given ATMN (0.5 mg/kg) 15 min later followed by oxotremorine (1 μg/kg, 5 rabbits), (10 μg/kg, 8 rabbits) or (40 μg/kg, 5 rabbits), and measurements of blood pressure, heart rate

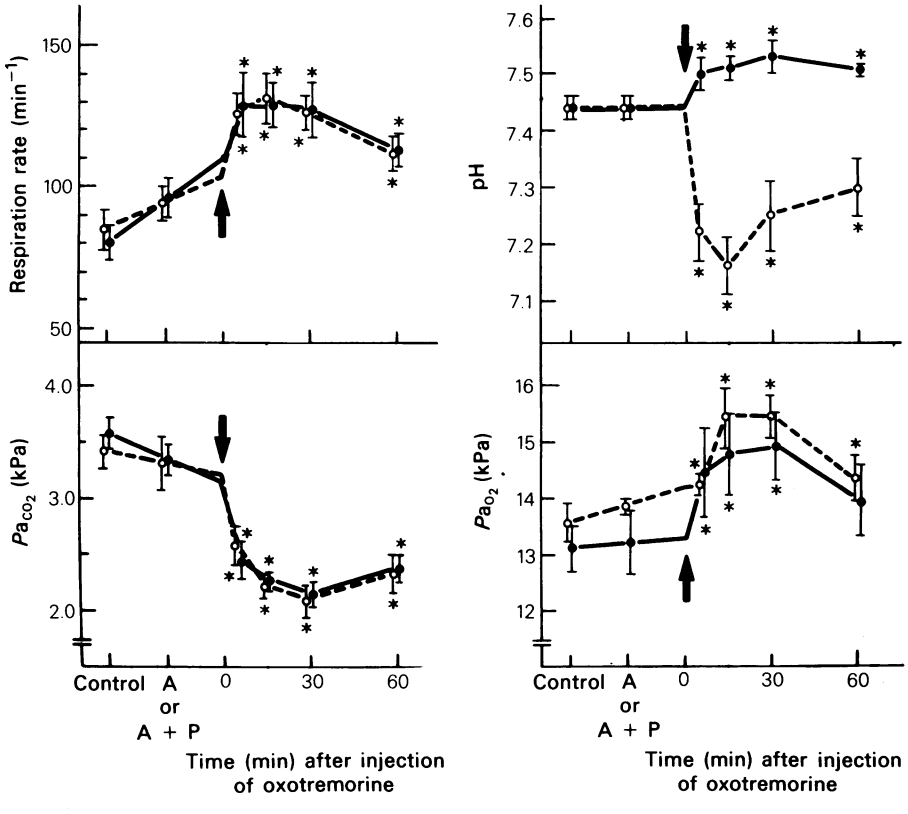

Figure 1 The effect of propranolol pretreatment on the respiratory stimulant effect of oxotremorine in the rabbit. (○) (Controls) rabbits pretreated with atropine-methyl-nitrate (ATMN) 0.5 mg/kg (A) 15 min before oxotremorine 10 μg/kg injected at arrow; (●) rabbits pretreated with propranolol 5 mg/kg s.c. (P) 30 min and ATMN 0.5 mg/kg (A) 15 min before oxotremorine 10 μg/kg injected at arrow.
*Significantly different from ATMN or ATMN + propranolol by paired *t* test.

and blood gases and pH were carried out at the times described above. Blood lactic acid concentrations were only determined in samples from rabbits given oxotremorine (10 μg/kg). Four other rabbits were pretreated with hyoscine (5 mg/kg) and then given oxotremorine (10 μg/kg) 15 min later. Measurements of blood pressure and blood gases and pH were made as described above.

The following drugs were used: atropine-methyl-nitrate (Sigma Ltd.), oxotremorine (Aldrich Ltd.), (±)-propranolol (Ayerst Ltd.), hyoscine hydrobromide (Endo Products, Inc., N.Y.).

All drugs were freshly prepared in sterile apyrogenic saline before each experiment. All doses are expressed in μg or mg/kg body weight of the salt.

Statistical analysis

Tests of significance for the difference between mean values were performed by Student's *t* test for paired or unpaired data as indicated in Results. Results were considered to be significantly different if the *P* value by this test was less than 0.05 and such a difference is indicated by an asterisk.

Results

Atropine methyl nitrate (0.5 mg/kg) caused a slight increase in respiration rate in control rabbits, but the changes in blood gases induced by this drug were not statistically significant (Figure 1).

Oxotremorine (10 μg/kg) further increased respiration rate from 94 ± 6 to 131 ± 9 per min at 15 min after injection, and reduced Pa_{CO_2} from 3.30 ± 0.23 kPa to 2.22 ± 0.10 kPa, while Pa_{O_2} was elevated from 13.83 ± 0.13 to 15.43 ± 0.53 kPa. These changes in respiration rate and blood gases lasted for more than 60 min (see Figure 1). At the same time, blood pH fell markedly from 7.44 ± 0.02 to 7.16 ± 0.05 at 15 min after injection of oxotremorine (Table 1 and Figure 1). Blood pressure rose by 11.6 ± 1.3%, 5 min after oxotremorine (Figure 2). All these effects of oxotremorine were prevented by pretreating the animals with hyoscine (5 mg/kg) (see Table 1).

Blood lactic acid levels increased from 41.4 ± 2.9 to 132 ± 13 mg/100 ml, 15 min after oxotremorine (see Figure 2). Pretreatment of rabbits with propranolol (5 mg/kg) completely prevented the fall in blood pH and converted it to 7.51 ± 0.02, a significant rise, after oxotremorine (see Figure 1). Propranolol caused a small but significant decrease in resting blood lactic acid levels, and markedly reduced the rise in lactic acid concentration after oxotremorine to a value that did not differ significantly from control rabbits given ATMN alone (see Figure 2). At the same time, propranolol did not decrease the rise in blood pressure (Figure 2), respiration rate or changes in blood gases induced by oxotremorine (10 μg/kg) (Figure 1).

It was possible to obtain a small but significant increase in respiration rate and decrease in Pa_{CO_2} with a dose of oxotremorine as low as 1 μg/kg (Figure 3). At a higher dose of 40 μg/kg, the increase in respiration rate was proportionally greater than the fall in Pa_{CO_2} compared with the changes in these parameters produced by oxotremorine (10 μg/kg) in propranolol pretreated rabbits.

Discussion

The present study demonstrates that oxotremorine can cause prolonged stimulation of respiration in the conscious animal. The increase in respiration rate and Pa_{O_2} and the fall in Pa_{CO_2} were due to stimulation of muscarinic receptors in the central nervous system since they were prevented by pretreatment with hyoscine but not by ATMN which does not readily penetrate the blood brain barrier (Herz, Teschamacher, Hoffstetter & Kurg, 1965).

This study also demonstrates the importance of

Table 1 The effect of hyoscine on the respiratory stimulant and pressor effect of oxotremorine

Treatment	n	*Respiration rate* (per/min)	*pH*	Pa_{CO_2} (KPa)	Pa_{O_2} (KPa)	*MABP*[a]
ATMN 0.5 mg/kg	8	94 ± 6	7.44 ± 0.02	3.30 ± 0.23	13.83 ± 0.13	75.2 ± 2.8
ATMN 0.5 mg/kg + oxotremorine 10 μg/kg	8	131* ± 9	7.16* ± 0.05	2.22* ± 0.10	15.43* ± 0.53	87.2* ± 2.2
Hyoscine 5 mg/kg	4	89 ± 9	7.43 ± 0.03	3.42 ± 0.21	13.67 ± 0.22	71.8 ± 3.4
Hyoscine 5 mg/kg + oxotremorine 10 μg/kg	4	91 ± 7	7.40 ± 0.03	3.32 ± 0.22	13.78 ± 0.23	73.3 ± 3.6

Results represent the mean ± s.e.mean 15 min after injection of all drugs for respiratory parameters and mean arterial blood pressure (MABP) after atropine-methyl-nitrate (ATMN) or hyoscine, and 5 min after injection of oxotremorine for MABP.

*Significantly different from ATMN alone by paired *t* test.

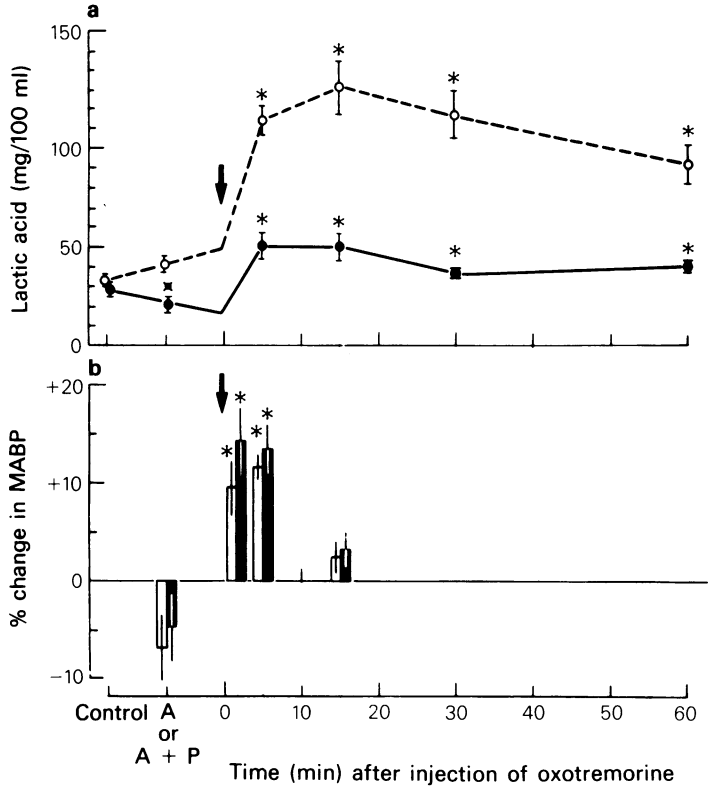

Figure 2 The effect of propranolol pretreatment on the lactic acidosis and blood pressure elevation induced by oxotremorine. (a) (○) (Controls) rabbits pretreated with atropine-methyl-nitrate (ATMN) 0.5 mg/kg (A) 15 min before oxotremorine 10 μg/kg injected at arrow; (●) rabbits pretreated with propranolol 5 mg/kg s.c. (P) 30 min and ATMN 0.5 mg/kg (A) 15 min before oxotremorine 10 μg/kg injected at arrow. (b) Open columns: controls as above. Solid columns: propranolol pretreated as above.
*Significantly different from control untreated rabbits or significantly different from ATMN or ATMN + propranolol.

measuring blood pH and blood gas tensions, in addition to changes in respiratory depth or frequency, in order to determine the mechanism of action of drugs on respiration. Thus, the blood pH fell dramatically from 7.44 to 7.2, 5 min after injection of oxotremorine, in spite of a considerable reduction in Pa_{CO_2}. The reduction in pH was found to be due to a marked elevation of blood lactic acid which occurred without any evidence of tremor or overt muscle activity. Since the lactic acidosis and pH change were also antagonized by hyoscine, the possibility was examined that the hyperventilation was secondary to the metabolic acidosis, which itself was initiated by activation of central muscarinic receptors.

In a previous study in rats, it was found that oxotremorine can stimulate muscarinic receptors in the central nervous system to bring about an activation of the sympatho-adrenal system and cause a 3–4 fold elevation of plasma catecholamines (Weinstock, Zavadil, Chieuh & Kopin, 1979). In the present experiments, there was also evidence of catecholamine release by oxotremorine as blood pressure increased significantly 2–5 min after its injection.

It is known that catecholamines can increase lactic acid production by stimulation of β-adrenoceptors in cardiac and skeletal muscle (Himms-Hagen, 1972). Therefore, it was likely that the lactic acidosis was caused by catecholamines released from the adrenal medulla and sympathetic nerves. This possibility was confirmed by the finding that propranolol prevented the increase in lactic acid by oxotremorine and converted the fall in pH to a significant rise. At the same time, propranolol did not reduce either the increase in respiratory rate and in Pa_{O_2} or the fall in Pa_{CO_2} produced by oxotremorine. One may therefore conclude that oxotremorine can stimulate respiration by a direct action on muscarinic receptors in the central nervous system.

This study also raises the possibility that other drugs which release adrenal catecholamines may influence blood pH and respiration directly by causing

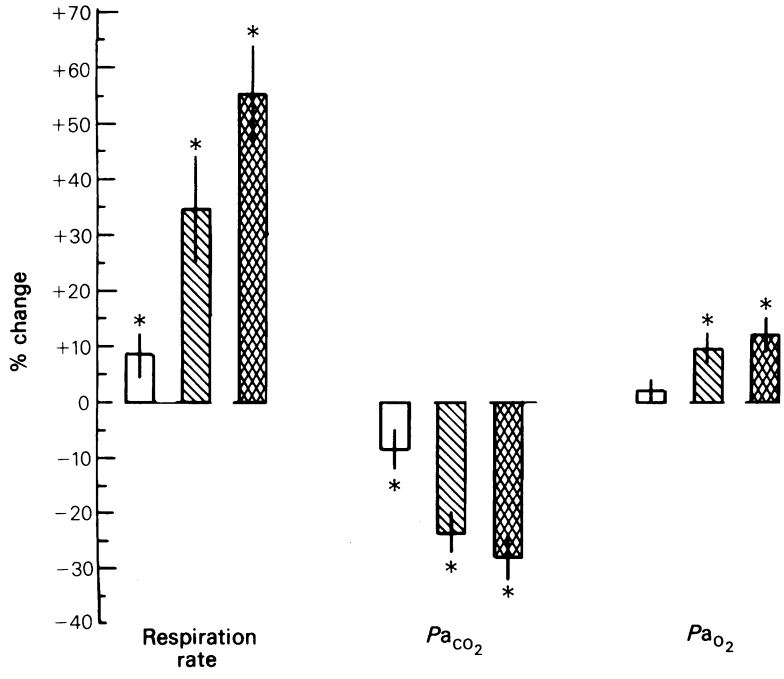

Figure 3 The effect of different doses of oxotremorine on respiration rate, Pa_{CO_2} and Pa_{O_2}. Open columns: oxotremorine 1 μg/kg at 30 min; hatched columns oxotremorine 10 μg/kg at 30 min; cross-hatched columns: oxotremorine 40 μg/kg at 30 min. *Significantly different from atropine-methyl-nitrate and propranolol by paired *t* test.

lactic acidosis. Such an action should be taken into consideration when examining the effects of drugs on respiration. It may be adequately controlled by β-adrenoceptor blockade.

A small but significant stimulant effect on respiration could be demonstrated with oxotremorine (1 μg/kg). At a higher dose (40 μg/kg) of oxotremorine, respiration rate was increased to values exceeding 200/min in propranolol pretreated animals, but the Pa_{CO_2} did not decrease proportionately to the value achieved after 10 μg/kg. Presumably at such a high rate of breathing, the depth becomes shallower and the dead space ventilation becomes disproportionately greater resulting in a less efficient gas exchange.

It is also apparent that oxotremorine can continue to cause hyperventilation for 60–90 min in spite of the fact that the Pa_{CO_2} has been decreased to 32% below normal values. It is well known that a lowering of Pa_{CO_2} decreases alveolar ventilation and thereby restores Pa_{CO_2} to control values under normal conditions (Lambertsen, 1980). Our findings suggest that cholinergic receptor activation can either bypass the control of respiration normally exerted by CO_2 on the respiratory centre or act independently of it. Alternatively, CO_2 may activate a cholinergic pathway which then acts on the respiratory centre.

The latter possibility is supported by the finding that local application of atropine to the ventral surface of the medulla reduced the ventilatory response to CO_2 (Dev & Loeschcke, 1979b).

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