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EFFECTS OF CHOLINOMIMETIC AGENTS GIVEN INTO THE BRAIN OF FOWLS

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1 Effects of cholinomimetic agents, given into the IIIrd ventricle of adult fowls (*Gallus domesticus*) or infused into the hypothalamus of young chicks, were tested on behaviour, respiratory rate and body temperature.

2 Carbachol evoked behavioural and electrocortical arousal but lacked postural and respiratory effects. Contrariwise, pilocarpine increased respiratory rate and induced postural changes, i.e. abduction of the wings, but lacked other behavioural effects and did not alter electrocortical activity. Benzoylcholine induced tachypnoea, postural changes and brief electrocortical arousal. Acetylcholine was ineffective unless given with physostigmine, when electrocortical arousal, postural changes and tachypnoea developed. Methacholine induced tachypnoea and postural changes.

3 Effects of carbachol and pilocarpine were prevented by hyoscine and those of benzoylcholine by pempidine; hyoscine and pempidine were required together to prevent the effects of methacholine and to attenuate those of acetylcholine with physostigmine.

Introduction

Cholinomimetic substances, three aliphatic (acetylcholine, acetyl-β-methacholine (methacholine), carbamylcholine (carbachol)) and two alicyclic (benzoylcholine and pilocarpine), were given into the IIIrd cerebral ventricle or infused into the hypothalamus of adult fowls to ascertain whether their central mode of action was muscarinic or nicotinic. Such categorization depended on whether their effects resembled those of muscarine or nicotine (see Marley & Seller, 1972, 1974) and whether these effects were prevented by hyoscine or by pempidine. Classification according to the pharmacological antagonist proved to be more practicable.

Methods

Techniques for injection or infusion of drugs into the IIIrd cerebral ventricle of adult fowls and the hypothalamus of young chicks, via implanted cannulae, have been described elsewhere (Marley & Stephenson, 1968, 1970; Grunden & Marley, 1970). Details of other techniques and recording methods used are given by Marley & Seller (1972). Experiments were performed at thermoneutrality, i.e. 29-31°C for chicks aged 12-21 days and 20-25°C for adult fowls. The volume of drinking water and weight of food in the test chamber were recorded before and after infusing carbachol into the hypothalamus.

Drugs

The drugs used were the hydrochlorides of acetylcholine, benzoylcholine, carbachol, methacholine and pilocarpine; also used were hyoscine hydrobromide and pempidine tartrate. Drugs injected intraventricularly or infused into the brain are expressed as μmol or nmol total dose; those administered intraperitoneally are given as μmol/kg for adult fowls and as μmol/100 g for young chickens.

Results

Antagonism by hyoscine

Carbachol. Intraventricular carbachol was given in 6 experiments on five adult hens; it was infused also into the brain of 26 young chickens.

Intraventricular injection. Carbachol (6 nmol) evoked behavioural and electrocortical arousal. The 'drowsy' electrocorticogram (120-250 μV, 6-8 Hz) changed immediately to an alert pattern (30-100 μV, 10-14 Hz) associated with a reduction of electrocortical integrals from 150-175/min to a

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minimum of 60/min; recovery occurred after 40 minutes. Behavioural arousal was marked, 'escape' responses being observed, but respiratory and postural changes did not develop. Repeated injections of carbachol had reproducible effects.

Hyoscine diminished the effects of carbachol. Thus, the duration of behavioural and electrocortical arousal evoked by carbachol (6 nmol) injected into a drowsy fowl 10 min after hyoscine (2 μmol/kg i.p.) was reduced to 15 minutes. However, the intensity of electrocortical arousal was unimpaired. Double the dose of carbachol (i.e. 12 nmol) induced behavioural and electrocortical arousal of similar intensity to that induced by the 6 nmol dose prior to hyoscine. Effects of carbachol (6 nmol) were undiminished by pempidine (2 μmol/kg i.p.).

Infusion into the hypothalamus. Carbachol (6 nmol) so infused into a drowsy chick evoked immediate behavioural and electrocortical (Fig. 1b) arousal associated with a reduction of electrocortical integrals (Figure 1d). Electrocortical activity changed from a 'drowsy' control pattern of large amplitude (70-150 μV) slow waves (4-8 Hz, Fig. 1a) to an intensely alert pattern (20-60 μV, 10-14 Hz, Fig. 1b), abating after 110 min (Figure 1c). During electrocortical alerting, the chick exhibited bouts of motor activity alternating with quiescence. Body temperature, food and water intake were unaffected.

Hyoscine prevented these effects of carbachol. Thus, hyoscine (0.5 and 5 μmol/100 g i.p.), itself without effect on behaviour, electrocortical activity and integrals of electrocortical activity in a drowsy chick (Fig. 1e), prevented the alerting effect of two doses of carbachol (each of 6 nmol), given 20 and 40 min subsequently. Pempidine (0.5 and 5 μmol/100 g i.p.) was ineffective against carbachol (6 nmol).

Pilocarpine

Intraventricular injection. Thirteen experiments were performed in seven adult fowls. Within 5 min of intraventricular pilocarpine (0.05 μmol) respiratory rate increased from control values (16-24/min) to 160-220/min (Fig. 2b), an effect lasting 90 min and accompanied by postural changes similar to those after intraventricular muscarine, i.e. abduction of wings from the trunk with lowering of the tail. However, unlike experiments with muscarine, electrocortical activity and integrals were unaltered (Figure 2a). On recovery, hyoscine (18.2 μmol/kg i.p.) was injected. Respiratory (Fig. 2b) and postural effects of pilocarpine (0.05 μmol) were prevented. Fifteen min later, a five-fold larger dose of pilocarpine (i.e.

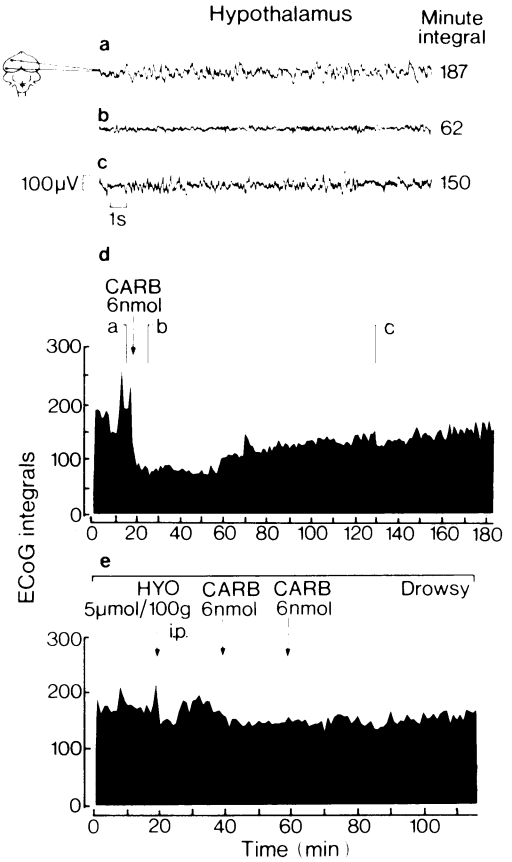

Fig. 1 Records of electrocortical activity (a to c) and histogram of integrated electrocortical activity (d and e) from unanaesthetized, unrestrained chicks; (a to d from a chick aged 16 days and e from a chick aged 13 days). Epochs corresponding to a, b and c are indicated in histogram d and integrals for the corresponding minute of electrocortical activity are given on the right of the traces. (a) Control drowsy electrocortical activity; (b) electrocortical arousal following infusion of carbachol (CARB; 6 nmol) into the lateral hypothalamus and associated with a decline of electrocortical integrals (d); (c) return of drowsy electrocortical activity; (e) lack of effect of two infusions of carbachol (each of 6 nmol) into the lateral hypothalamus following hyoscine (HYO; i.p.).

0.25 μmol) surmounted antagonism, although respiratory (Fig. 2b) and postural effects were less than those with the first dose of pilocarpine. As shown in Fig. 2c, tachypnoea evoked by intraventricular pilocarpine (0.05 μmol) was undiminished by pempidine (20 μmol/kg i.p.); postural effects were also unaltered.

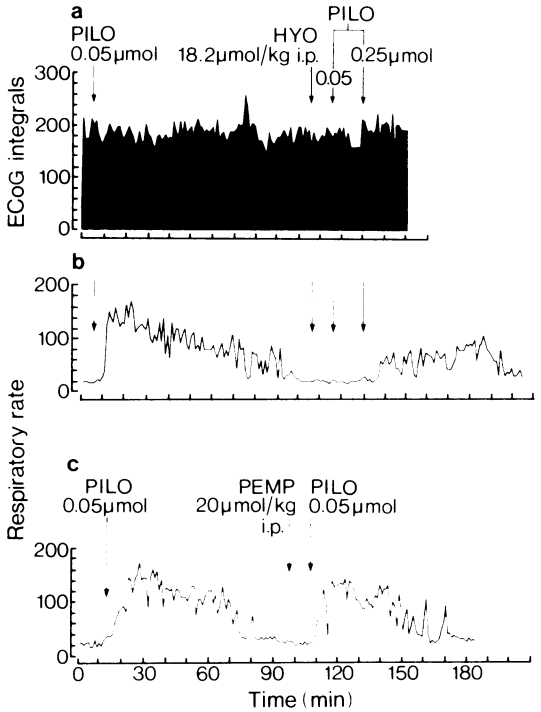

Fig. 2 Histogram of integrated electrocortical activity (a) and graphs of respiratory rates (b and c) in two unanaesthetized, unrestrained adult fowls (the one a and b, and the other c). (a) Lack of effect of pilocarpine (PILO) and hyoscine (HYO) on electrocortical integrals; (b) increase in respiratory rate after pilocarpine (0.05 μmol), but antagonism with hyoscine; antagonism surmounted by pilocarpine (0.25 μmol); (c) pempidine (PEMP) lacked significant antagonistic action on respiratory effects of pilocarpine (0.05 μmol).

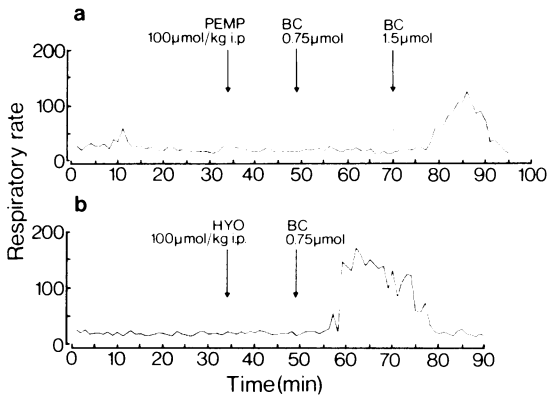

Fig. 3 Graphs of respiratory rate in two (a and b respectively) unanaesthetized, unrestrained fowls. (b) Increase in respiratory rate after benzoylcholine (BC; 0.75 μmol) occurs after hyoscine (HYO) but (a) is absent after pempidine (PEMP). Antagonism was surmounted by benzoylcholine (1.5 μmol).

Antagonism by pempidine

Benzoylcholine

Intraventricular injection. Intraventricular benzoylcholine was given in 15 experiments on ten adult fowls. Benzoylcholine (0.5 and 0.75 μmol) elicited brief electrocortical arousal, together with tachypnoea and postural changes similar to those elicited by nicotine. These effects were prevented by pempidine but not by hyoscine. As shown in Fig. 3b, 15 min after benzoylcholine, respiratory rate increased from control values (16-28/min) to a peak of 174/min (Fig. 3b) despite prior injection of hyoscine (100 μmol/kg i.p.). In contrast, after the same dose of pempidine, benzoylcholine (0.75 μmol) was ineffective (Fig. 3a), but the antagonism was surmounted by double the initial dose of benzoylcholine (i.e. 1.5 μmol; Fig. 3a), respiratory rate mounting to a peak of 128/min accompanied by postural changes. Both effects abated after 18 minutes.

Antagonism by hyoscine together with pempidine

Acetylcholine

Intraventricular injection. Acetylcholine was tested in 15 experiments on 11 fowls. Acetylcholine (0.2 μmol) alone did not alter respiratory rate and had minimal electrocortical effects. It was given, therefore, after intraventricular physostigmine (6 nmol) a dose lacking significant effects on respiratory rate, although inducing mild electrocortical arousal. Following physostigmine, intraventricular acetylcholine (0.11 μmol) now induced electrocortical alerting for 7 min; as the electrocortical effects abated, tachypnoea lasting 10 min developed with a peak rate of 68/min and the wings were abducted from the trunk. When one-fifth of this dose, i.e. acetylcholine (0.022 μmol), was subsequently injected intraventricularly together with physostigmine (6 nmol), the effects of acetylcholine were further intensified. Thus, respiratory rate increased to a peak of 160/min with recovery after 23 minutes. Electrocortical arousal was more marked, electrocortical integrals declining from 120-220/min to 36-45/min, an effect lasting 23 minutes. Because physostigmine itself caused electrocortical arousal, this intensified electro-

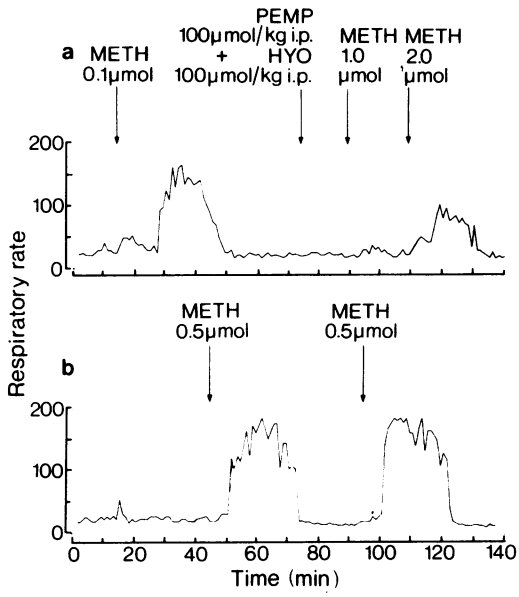

Fig. 4 Graphs of respiratory rate in two adult unanaesthetized, unrestrained fowls. In trace a, the effect of intraventricular methacholine (METH) is prevented by a combined dose of hyoscine (HYO; i.p.) and pempidine (PEMP; i.p.). Antagonism is partly surmounted by methacholine (2.0 μmol); (b) illustrates reproducibility of respiratory effects of intraventricular methacholine (0.5 μmol), the second dose given 50 min after the first.

cortical effect could be as readily attributed to summation as to potentiation.

In 4 experiments on three fowls, respiratory effects of intraventricular acetylcholine (0.022 μmol) together with physostigmine (6 nmol) were unaffected by 10 or 100 μmol/kg i.p. doses of hyoscine or pempidine given 12 min previously. However, in 2 further experiments, tachypnoea induced by this dose of acetylcholine combined with physostigmine was reduced in duration and attenuated 25% in intensity by hyoscine and pempidine given together (each in 10 μmol/kg i.p. doses).

Methacholine

Intraventricular injection. Twenty-five experiments were performed on nine adult fowls. Respiratory and postural changes developed but electrocortical effects were evanescent.

Reproducible increases in respiratory rate were obtained after methacholine (0.5 μmol; Fig. 4b) together with postural changes similar to those after intraventricular muscarine. There was a delay of 10 to 15 min before the onset of tachypnoea when the respiratory rate increased from 20-30/min over the ensuing 5 to 10 min to subsequent peaks between 100 and 200/min (Figure 4b). The termination of tachypnoea tended to be abrupt, in contrast to experiments with muscarine or nicotine in which it was gradual. Whereas prior dosage with pempidine (100 μmol/kg i.p.) only partially diminished the respiratory and postural effects of methacholine and hyoscine was ineffective, a combination of pempidine and hyoscine prevented these phenomena. Thus, methacholine (1.0 μmol) induced tachypnoea 13 min after injection, respiratory rate increasing to a maximum of 184/min with recovery after 24 min (Figure 4a). Twenty-four min later, pempidine and hyoscine were injected, each in doses of 100 μmol/kg intraperitoneally. This combination prevented the effects of methacholine (1.0 μmol) given 15 min later; the antagonism was partly surmounted by methacholine (2.0 μmol) which increased respiratory rate to 100/min for 20 min (Figure 4a).

Discussion

Peripheral effects of cholinomimetic substances are usually analysed in terms of their resemblance to those of muscarine and nicotine. We have attempted to apply this yardstick to the central effects of cholinomimetic substances. Such an analysis was not possible, since the cholinomimetic agents induced only a porportion of the phenomena elicited by muscarine and nicotine. For example, carbachol elicited behavioural and electrocortical arousal similar to muscarine but lacked its postural and respiratory effects. Contrariwise, pilocarpine induced respiratory and postural changes similar to muscarine but was without its behavioural or electrocortical effects. Benzoylcholine, with predominantly nicotine-like properties (Simonart, 1932), induced appropriate postural and respiratory changes but failed to elicit the initial sedative behavioural and electrocortical effects of nicotine.

Classification according to susceptibility to antagonists also presented problems. Thus, as expected, the effects of pilocarpine were prevented by hyoscine and those of benzoylcholine by pempidine. However, the electrocortical effects of carbachol, a substance with muscarinic and nicotinic properties, were prevented by hyoscine alone, whereas hyoscine and pempidine were required to antagonize the effects of methacholine, although the latter has marked muscarinic actions but weak nicotinic

properties. The effects of acetylcholine were only partly attenuated, even by hyoscine and pempidine together. This could have been due to mixed muscarinic and nicotinic actions of acetylcholine or, since it was necessary to combine physostigmine with acetylcholine, to an action also of physostigmine on neurones. Certainly, physostigmine applied iontophoretically excites cat brain-stem neurones which are unaffected by acetylcholine (Salmoiraghi & Steiner, 1963; Bradley, Dhawan & Wolstencroft, 1966), implying actions unrelated to its anticholinesterase property. Interactions of physostigmine with the cholinoceptive antagonists could also have complicated the interpretation. For example, cortical release of acetylcholine induced in cats by intravenous physostigmine was reduced by prior dosage with scopolamine but enhanced by mecamylamine (Bartolini, Bartolini & Domino, 1973).

Clearly, experiments involving cholinomimetic agonists and their antagonists, given after physostigmine, are difficult to interpret. Such problems arose in explaining prolongation by physostigmine of sleep-like behavioural and electrocortical activity induced by nicotine (Marley & Seller, 1974) when, not only did acetylcholine under similar circumstances evoke arousal, but the response was difficult to attenuate with cholinoceptive antagonists given singly or in combination.

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