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EFFECTS OF NICOTINE GIVEN INTO THE BRAIN OF FOWLS

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1 The effects of nicotine, given into the IIIrd ventricle of adult conscious fowls *(Gallus domesticus)* or infused into various brain regions of conscious young chicks, were tested on behaviour, electrocortical activity, respiratory rate and body temperature. Its effects given intraventricularly or applied externally to the brain-stem of anaesthetized fowls were also examined.

2 After intraventricular nicotine, fowls squatted for 3 to 5 min with eyes closed, electrocortical activity resembling that during sleep but with superimposed spike activity. Following this, fowls reawakened and tachypnoea developed, together with partial abduction of the wings from the trunk, the back becoming horizontal and the tail flexed. These effects were prevented by pempidine.

3 Intraventricular nicotine suppressed or, less commonly, reduced operant key-pecking, an effect unrelated linearly to dose.

4 Intraventricular nicotine given to fowls anaesthetized with chloralose produced brief apnoea, followed by increased amplitude of respiratory excursion for about 5 minutes. Respiratory rate accelerated slightly but tachypnoea did not develop. Nicotine applied directly to the ventral brain-stem increased respiratory amplitude in three out of seven fowls.

5 In anaesthetized fowls, intraventricular nicotine raised blood pressure for 2 to 3 min, an effect prolonged up to 70 min by acute bilateral vagotomy, whereas pressor effects of intravenous nicotine were extended merely two to three fold. Dividing the spinal cord at C2 prevented pressor effects of intraventricular nicotine; those of intravenous nicotine were unaltered.

6 In young chicks, nicotine infused into the diencephalon, telencephalon and myelencephalon induced effects similar to those observed immediately after intraventricular nicotine, i.e. chicks squatted with closed eyes but recovered within 3 to 5 minutes. Simultaneously, electrocortical activity changed from an alert to the sleep pattern, usually with superimposed 'spike' activity. Tachypnoea and associated postural changes did not develop. Pempidine prevented the behavioural and electrocortical effects of nicotine.

Introduction

The effects of nicotine given peripherally have been extensively investigated and a direct central action often inferred from such studies. However, interpretation is complicated, for although nicotine crosses the blood-brain barrier (Schmiterlöw & Hansson, 1965), it also elicits secretion of vasopressin and adrenal medullary catecholamines, substances with intrinsic central actions. The possibility of a direct central action of nicotine was indicated by results of Knapp & Domino (1962, 1963). In dogs with mid-pontine transection, a preparation which exhibits stable sleep activity, intravenous nicotine induced electrocortical arousal; adrenaline, noradrenaline and vasopressin, in doses sufficient to have marked pressor effects, were however without effect on electrocortical activity.

A series of papers concerning nicotine injected into the cerebral ventricles of cats (Armitage, Milton & Morrison, 1966; Hall & Reit, 1966; Armitage & Hall, 1967a,b; Armitage, Hall, Milton & Morrison, 1967; Schaeppi, 1967, 1968; Hall, 1968) provided evidence for a variety of central actions, while iontophoretic application of nicotine demonstrated its activity on neurones of different parts of the brain (Krnjević & Phillis, 1963; Bradley & Wolstencroft, 1965; Bradley, Dhawan & Wolstencroft, 1966).

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This paper presents results with fowls in which nicotine was infused into discrete areas of the brain and injected into the IIIrd cerebral ventricle; a brief account has been published (Marley & Seller, 1970a). The results indicate that not only can the central effects of nicotine be distinguished from those of muscarine but the selective antagonists for the substances differ, that for nicotine being pempidine whereas those for muscarine were atropine or hyoscine (Marley & Seller, 1970a,b; 1972).

Methods

Animals

Adult or young Rhode Island Red pullets were used. Adults (1.75-2.25 kg) were kept at room temperature (20°-22° C). Young chicks (12-21 days old, approximately 85 g) were maintained at a cage temperature of 20°-31° C.

Operative procedures

Stereotactic implantation of intraventricular and intracerebral cannulae, anaesthesia (halothane for recovery anaesthesia, chloralose for non-recovery experiments), postoperative care, methods for recording and integrating electrocortical activity, for recording blood pressure, body temperature and respiration, for injections via implanted cannulae, for histological preparation of the brain, were as described previously (Marley & Seller, 1972). Information not there detailed, includes:

Operant key-pecking. Tests were performed with adult fowls using a Grason Stadler pigeon-station in a sound-proof chamber. Fowls were kept on a diet, supplemented with weekly Parenterovite injections, which maintained them at about 75% of free-feeding weight. Training and testing were as described by Marley & Morse (1966) for young chickens. Of schedules used, the most sustained performance was achieved with a Variable Ratio; this consisted of a cycle of 231 pecks with eight food reinforcements, e.g. after 11, 36, 44, 71, 173, 198, 206 and 231 pecks, at which time the cycle recommenced.

Direct application of nicotine to the brain-stem. Approach to the brain-stem was by drilling through the basal part of the temporal bone, a procedure similar to that described by Schooley (1939) for hypophysectomy in pigeons. To obtain adequate exposure of the brain-stem, the internal carotid arteries which traverse the bone were ligated prior to drilling. Once the dura was reached, it was incised. Nicotine, in various final concentrations contained in 0.5 ml of 0.9% w/v NaCl solution (saline), was injected on to the brain-stem through the trephine hole, the fowls being supine. The drug remained in contact with the brain-stem for 5 min and was then replaced by saline; such doses were given at 60 min intervals. At termination of experiments, cresyl-fast violet was applied to the exposed area of brain-stem; the head was then removed and frozen. The area of dye-stained brain-stem, located by subsequent removal of the cranium, was taken as that which had been exposed to nicotine.

Drugs used were atropine sulphate, hyoscine hydrobromide, nicotine base, pempidine tartrate and physostigmine sulphate. Drugs applied directly to the brain-stem, infused into the brain or injected into the IIIrd cerebral ventricle, are expressed as μmol total dose.

Results

Intraventricular injections

Non-anaesthetized fowls. One hundred-and-three experiments were performed in 30 adult fowls; of these, 50 experiments were undertaken in nine fowls trained in operant key-pecking. Intraventricular nicotine produced characteristic and, provided the doses were spaced sufficiently, reproducible alterations in behaviour, posture, electrocortical activity and respiratory rate.

Posture and non-learnt behaviour. Effects of nicotine (0.5 μmol) all developed within 5 minutes. First, the fowls subsided to a squatting position; as they subsided, head scratching frequently occurred. They remained squatting for 3 to 5 min, during which time the eyes were usually closed. The animals then stood with eyes open, the wings being abducted from the trunk. The wing abduction, together with altered disposition of the wing feathers, were similar to those observed after muscarine (Marley & Seller, 1972). The tail was extended in contrast to muscarine experiments in which it was flexed. These postural changes were maximal some 10 min after the injection and abated after 30 to 40 minutes. They developed and declined *pari passu* with effects on respiratory rate.

Electrocortical activity. After nicotine (0.5 μmol) and during the period in which the fowl was squatting, the control alert electrocortical pattern (10-14 Hz, 50-100 μV, Fig. 1a) changed almost immediately to slow frequency (6-8 Hz), large amplitude (150-240 μV) potentials

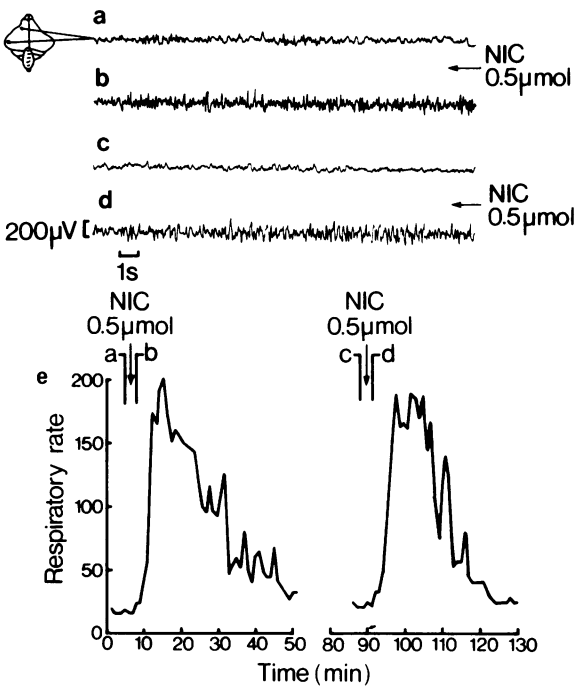

Fig. 1 Records of electrocortical activity (a to d) and graph of respiratory rate (e) in an unanaesthetized, unrestrained adult fowl. Epochs corresponding to a, b, c and d are indicated in the graph of respiratory rate. (a and c) Control alert electrocortical activity; (b and d) large amplitude 6-8 Hz electrocortical activity with superimposed 'spike' discharge following intraventricular nicotine (NIC, 0.5 μmol); (e) increase of respiratory rate associated with the two injections of nicotine. The second dose had effects of similar magnitude to the first, given 85 min previously.

lasting 3 to 4 min (Figure 1b). These were only distinguishable from sleep electrocortical activity by an irregular superimposed 'spike' discharge. Similar effects were obtained with the same dose of nicotine given 83 min later (Figure 1d).

Respiration. Nicotine (0.5 μmol) increased respiratory rate (Fig. 1e) which rose rapidly from a control of 14-24/min to 200/min, the effect gradually subsiding over 25 minutes. Whereas nicotine (0.0625 μmol) was ineffective on respiration, a dose of 0.125 μmol increased respiratory rate from control values to 128/min (Fig. 3) an effect lasting 25 minutes. In contrast to the delay with muscarine, respiratory effects of nicotine developed within 5 minutes.

Body temperature. Nicotine (0.5 μmol) did not alter body temperature at thermoneutrality.

Learnt behaviour: operant pecking. Nicotine reduced or suppressed operant pecking, effective doses being smaller than those altering non-learnt behaviour.

Thus, while intraventricular saline scarcely affected pecking (Fig. 2a), a subsequent intraventricular dose of nicotine (0.0625 μmol) stopped pecking for 26.4 min (Figure 2a and b). During this time the fowl was alert and squatting; thereafter, it stood and recommenced pecking at about the control rate (Figure 2b). Effects in a fowl more resistant to nicotine are shown in Fig. 2c and d; as before, intraventricular saline did not alter pecking (Figure 2c). Nicotine (0.125 μmol) stopped pecking for 2.9 min during which the fowl squatted but was alert; when pecking recommenced, the rate was not significantly different from that for the control period (Figure 2c). Double the dose of nicotine, i.e. 0.25 μmol, slowed pecking for 4.75 min following which pecking stopped for 1.5 min (Figure 2d); pecking then recovered but was slower than the control rate.

Potentiation. The sleep-like behavioural and electrocortical effects of nicotine, together with those on respiratory rate (Fig. 3) and posture, were potentiated by physostigmine. Nicotine (0.125 μmol) raised respiratory rate from control values to a maximum of 128/min, the effect starting 6 min after injection (Fig. 3a) and lasting 22 minutes. Following physostigmine (0.75 μmol/kg i.p.), a dose without effect on respiration, nicotine (0.125 μmol) now raised respiratory rate from control values to 170/min, the effect starting immediately after injection and lasting 24 min (Figure 3b). A dose of nicotine with maximal effects on respiratory rate was not potentiated in intensity by physostigmine (0.4 μmol/kg i.v.) but latency for onset of tachypnoea was shortened from 7 to 3 min and its duration prolonged from 28 to 33 minutes.

Tachyphylaxis. Effects of nicotine were reproducible so long as it was administered at intervals of 40 min or greater. At shorter intervals, tachyphylaxis developed, an effect clearest on respiration although electrocortical and behavioural changes also dwindled. Thus, a first dose of nicotine (0.25 μmol) increased respiratory rate from 25 to 190-200/min, an effect lasting 20 minutes. On return of respiratory rate to the pre-injection condition, a second dose of nicotine (0.25 μmol) was given; this was now ineffective. Doubling the nicotine dose to 0.5 μmol increased respiratory rate for 20 min to a maximum of 105/minute. However, effects of a fourth dose of nicotine (0.5 μmol) given 30 min later, were much

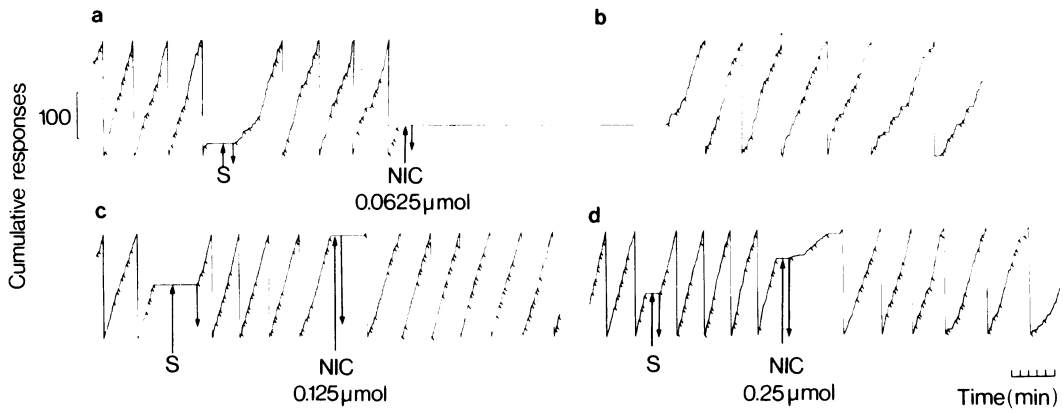

Fig. 2 Cumulative pecking records showing the suppressant effects of nicotine (i.v.) in two fowls performing on a Variable Ratio schedule. The short diagonal lines on the records indicate presentations of the food reinforcer. At upward pointing arrow, fowl removed from chamber and intraventricular injection of saline (S) or nicotine (NIC) given; at downward pointing arrow, fowl returned to chamber, following injection. In first fowl, complete suppression of pecking followed nicotine (a and b) with abrupt complete recovery (b). In second fowl, nicotine (0.125 μmol) briefly suppressed pecking (2.9 min) with sudden complete recovery (c), whereas nicotine (0.25 μmol) slowed pecking for 4.75 min, followed by cessation for 1.5 min and then abrupt recovery.

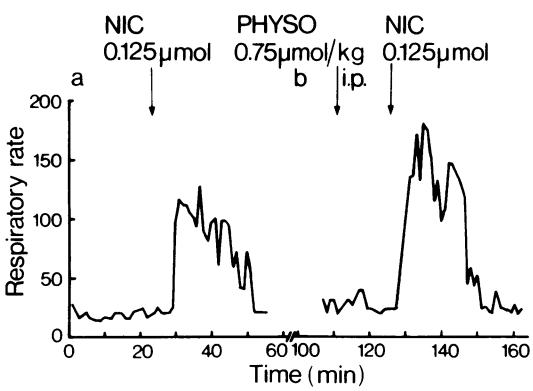

Fig. 3 Graphs of respiratory rate in an unanaesthetized unrestrained adult fowl to show potentiation of a submaximal effect of nicotine (NIC) by physostigmine (PHYSO). (c) Effects on respiratory rate of intraventricular nicotine (0.125 μmol). On recovery, (b) physostigmine (0.75 μmol/kg i.p.) was injected; effects of intraventricular nicotine (0.125 μmol) on respiratory rate were now much more marked.

attenuated, respiratory rate only twice exceeding 60/min in the ensuing 30 minutes. A fifth dose of nicotine (0.5 μmol), 25 min after the fourth, briefly raised respiratory rate to 50/minute. The final injection of nicotine (2.5 μmol) could have induced fatal convulsions in an untreated fowl. Instead, and after a delay of 15 min, respiratory rate rose for 45-50 min, from 20-30/min to a maximum of 200/minute. The decline in nicotine's effect could only be minimally attributed to unresponsiveness of the brain caused by the large total volume (60.0 μl) injected into the ventricle with the six nicotine doses (each of 10 μl). This was because the respiratory effects of the second of two intraventricular doses of nicotine, each of 0.25 μmol and 10 μl volume, was unimpaired following four spaced intervening intraventricular injections of saline each of 10 μl.

Antagonism. Electrocortical, behavioural and respiratory effects of nicotine were prevented by intraperitoneal doses of pempidine or mecamylamine but not of hyoscine or atropine. Thus, nicotine (0.5 μmol) converted the alert electrocorticogram (Fig. 4a) to a large amplitude 'spiky' 7-9 Hz activity (Fig. 4b), reverting to an alert pattern within 5 min (Fig. 4c); respiratory rate rose from 20/min to 184/min (Fig. 4f, left trace) with return to pre-injection values 35 min later. Pempidine (50 μmol/kg i.v.), itself without electrocortical or respiratory effects, was injected 60 min after the first nicotine dose. Nicotine (0.5 μmol, given intraventricularly) was now ineffective on electrocortical activity (compare Fig. 4e with d), and although respiratory rate still rose to a maximum of 170/min, duration of effect was more than halved to 15 min (Fig. 4f, right

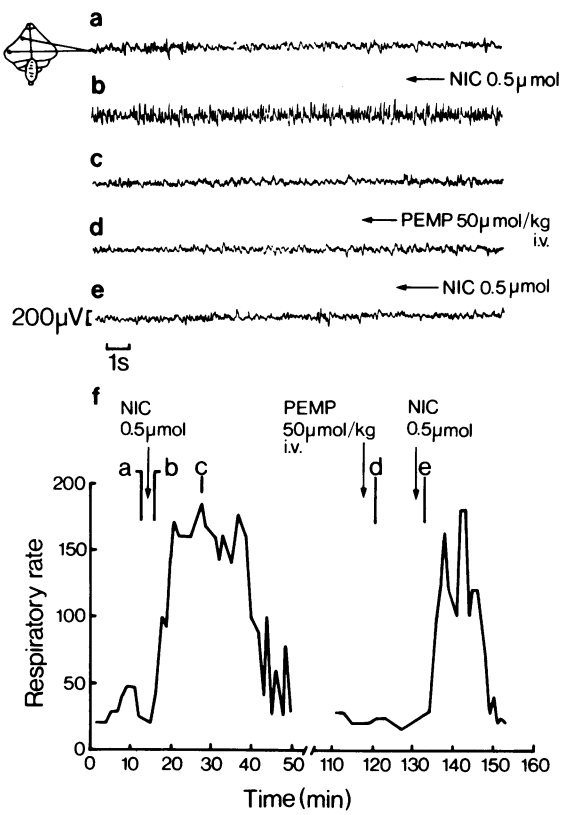

Fig. 4 Records of electrocortical activity (a to e) and graph of respiratory rate (f) in an unanaesthetized adult fowl. Epochs corresponding to a, b, c, d and e are indicated in the graph of respiratory rate. (a and c) Control alert electrocortical activity; (b) 'spiky' large amplitude 7-9Hz electrocortical activity after nicotine (NIC; 0.5 μmol) intraventricularly; (d) alert electrocortical activity unaltered by pempidine (PEMP); (e) electrocortical effects of nicotine abolished by pempidine; (f) considerable reduction by pempidine in duration of respiratory effects of nicotine.

trace). Double the dose of pempidine (100 μmol/kg i.p.), completely suppressed the effects of nicotine (0.5 μmol), while nicotine 1.0 μmol had only minimal respiratory and postural effects. In contrast, hyoscine (100 μmol/kg i.p.) did not antagonize respiratory, behavioural and electrocortical effects of nicotine.

While pempidine given intravenously or intraperitoneally did not affect respiratory rate, intraventricular pempidine (1.0 μmol) increased respiratory rate, reaching a peak of 130/min with recovery after 40 minutes.

Anaesthetized fowls

Respiration and blood pressure. Nine fowls were tested. Within 45 s of intraventricular nicotine (0.5 μmol), blood pressure rose from 110 to 180 mmHg, accompanied by brief apnoea, with return to pre-injection pressure after 2 min (Figure 5a). Apnoea was followed by increased respiratory amplitude for 3 min; respiratory rate increased from 18/min to 25/minute. These effects were reproducible when nicotine injections were spaced at least 60 min apart. Prolonged increase in respiratory rate 5 min after injection, noted with nicotine in non-anaesthetized fowls, did not occur. Pressor and respiratory effects of nicotine were little affected by hyoscine (60 μmol/kg i.v., Fig. 5b), but prevented by pempidine (50 μmol/kg i.v.). After acute division of the vagi in the neck, pressor effects of intraventricular nicotine were much prolonged. Thus in the experiment from which Fig. 5a was derived, following bilateral vagotomy, intraventricular nicotine (0.5 μmol) raised blood pressure from 80 to 170 mmHg (Fig. 5c), an effect maintained 30 min later (Fig. 5d), with return to pre-injection values after 75 min (Figure 5e). After spinal cord division at C2, intraventricular nicotine (1.0 μmol) was ineffective on blood pressure (Fig. 5f), indicating its pressor effect was mediated via the brain-stem and spinal cord.

In five fowls, effects of intravenous and intraventricular nicotine were compared, to ensure the latter were not due to leak of nicotine into the systemic circulation. Effects differed in a number of ways. Thus, intravenous nicotine even in larger doses (0.5 μmol/kg) had smaller pressor effects; blood pressure rose 30 to 40 mmHg for 1 to 2 min and respiratory rate slowed rather than accelerated following the apnoeic period. After acute bilateral vagotomy, pressor effects of intravenous nicotine (0.5 μmol and 0.75 μmol/kg) were prolonged merely to between 4 and 7 min; after division of the spinal cord at C2, pressor effects of intravenous nicotine were little altered.

Application of nicotine to the brain-stem. Blood pressure and respiration were recorded in seven fowls lightly anaesthetized with chloralose. Effective doses of nicotine were 0.25 and 0.5 μmol, except in one fowl in which respiratory changes were obtained with 0.04 μmol. In four fowls, nicotine (0.25 to 0.5 μmol) caused within 2 to 5 min clonic jerking of the trunk and limbs lasting up to 30 min, together with shorter-lived repetitive mandibular movements. In three fowls, nicotine (0.04 and 0.25 μmol) increased respiratory amplitude 50 to 100%, in one, during the 5 min of drug application and, in two, within 2

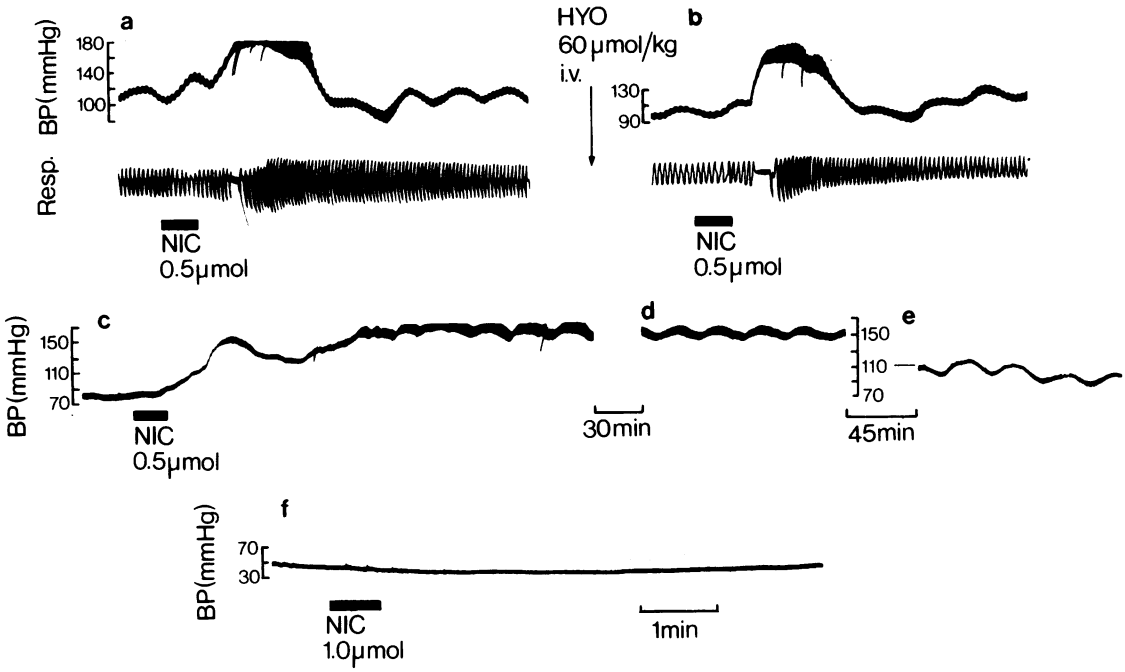

Fig. 5 Records of blood pressure (BP) and respiration (RESP) in a chloralose anaesthetized adult fowl. In a and b, the vagi were intact. In the respiratory trace, a complete upstroke and downstroke represents inspiration and expiration. In c to e, the vagi had been divided in the neck (bilateral vagotomy), the fowl being artificially ventilated. In f, there was additional spinal cord transection. The black bars indicate duration and timing of intraventricular injections. (a) Rise in blood pressure after nicotine (0.5 µmol intraventricularly) associated with brief apnoea and followed by increased respiratory amplitude and rate for 4 minutes; (b) effects of nicotine little altered by prior dose of hyoscine (HYO); (c) much prolonged effect of intraventricular nicotine after bilateral vagotomy; (f) effects of intraventricular nicotine on blood pressure abolished after spinal cord division at C2.

to 5 min of replacing nicotine by saline. Respiratory rate was unaltered, except in one fowl 12 min after nicotine (0.5 µmol) when respiration slowed from 23 to 6/min and then ceased; artificial ventilation was begun and spontaneous respiration returned 2 min later. In one fowl, blood pressure rose from 80 to 160 mmHg within 10 s of applying nicotine (0.25 µmol) but returned to 90 mmHg 1 min later. Whether nicotine produced effects or not, the area of drug application was found post-mortem to extend on the ventral and ventro-lateral surfaces of the brain-stem bounded anteriorly by the VIth and posteriorly by the IXth cranial nerve roots.

Intracerebral infusions

Forty-six experiments were performed in 20 chicks (12 to 21 days old). Reproducible behavioural and electrocortical changes were observed similar to those that developed immediately after intraventricular injection of nicotine in adult fowls.

Infusion into the diencephalon. Effects of nicotine infused into the hypothalamus on behaviour and posture are shown in Figure 6. Thus, an alert chick (Fig. 6a) immediately after infusion of nicotine (0.125 µmol) into the hypothalamus, squatted (Fig. 6b) with closed eyes. Although not evident from this Figure, the wings were usually abducted from the trunk. Some recovery was evident 3 min later (Fig. 6c), the chick partly standing full recovery occurred by 5 minutes. Electrocortical changes were similar to those in Figure 8b. Thus, after nicotine (0.125 µmol), the alert electrocortical pattern changed to 5-6Hz, 100-200 µV activity with bilateral superimposed 'spikes'; recovery occurred after 6 minutes. Postural and respiratory changes, which developed 5 min after intraventricular

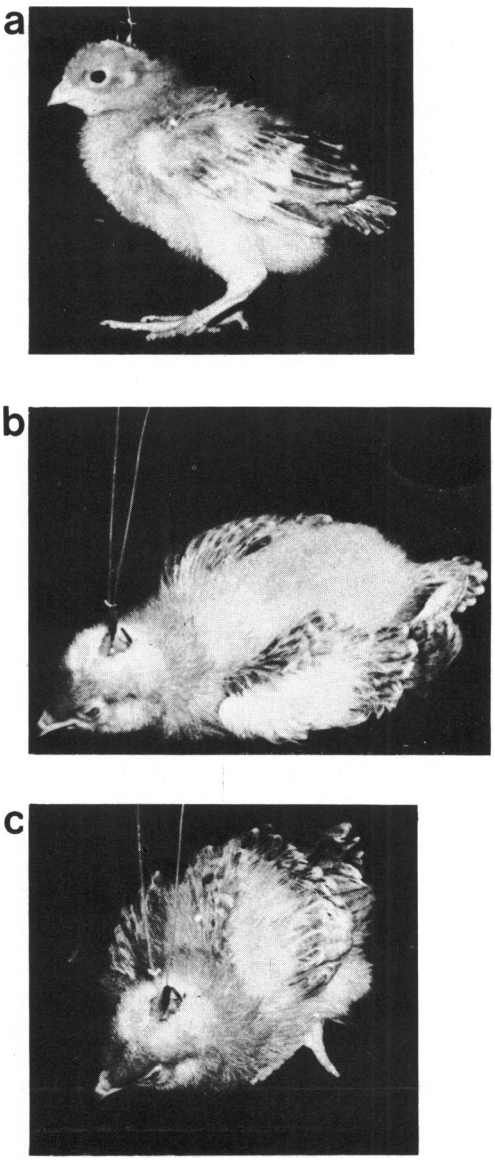

Fig. 6 Effects on posture of nicotine (0.125 μmol) infused into the hypothalamus of a 16 day chick. (a) Control alert; (b) within 30 s of infusing nicotine, the chicken squats, the wings are lowered, the beak rests on the ground and the eyes are closed; (c) 2 to 3 min after nicotine the chicken is standing, the wings are no longer lowered but the eyes remain closed.

nicotine and lasted 30 to 40 min, were not observed. Body temperature was unaltered.

Infusion into the telencephalon. Nicotine (0.125 μmol) infused into telencephalic sites, i.e. the paleostriatum augmentatum medial to the paleostriatum primitivum, the neostriatum and the anterior telencephalon close to the olfactory bulb, evoked similar behavioural and electrocortical changes to those described for diencephalic infusions, except that superimposed electrocortical 'spikes' were confined to the ipsilateral cerebral hemisphere, activity from the contralateral hemisphere being of the sleep pattern.

Infusion into the myelencephalon. When infused into the myelencephalon dorsal to the nucleus linearis caudalis in three chicks, nicotine evoked identical but more profound behavioural effects than those obtained with diencephalic or telencephalic infusions; bilateral electrocortical changes were also elicited. Thus, immediately after infusion of nicotine (0.125 μmol), the chick squatted with closed eyes and abducted wings; recovery occurred after 25 minutes. This dose of nicotine changed the alert electrocortical pattern (Fig. 7a) to slow frequency (6-8 Hz), large amplitude (120-250 μV) potentials with superimposed 'spike' discharge (Fig. 7b), 25 min elapsing before electrocortical activity returned to the pre-injection pattern. The more profound and long-lasting electrocortical effects of nicotine were reflected in the substantial rise of integrals (Figure 7d).

Antagonism. Behavioural and electrocortical effects of nicotine infused into the brain were prevented by systemic or local administration of pempidine but not hyoscine. Thus, alert electrocortical activity and behaviour were unaffected by hyoscine (10 μmol/100 g i.v.) as were electrocortical and behavioural effects of nicotine (0.125 μmol) infused into the hypothalamus 20 min subsequently. In the same chicken 24 h later, an identical dose (10 μmol/100 g i.v.) of pempidine given 20 min previously had no effect on alert electrocortical activity but prevented the effects of two (three, in other experiments) doses of nicotine each of 0.125 μmol infused into the hypothalamus.

These results were confirmed in experiments in which the antagonist was infused into the hypothalamus, 20 min before the agonist. Thus, hyoscine (0.15 μmol) neither affected alert electrocortical activity (Fig. 8a) and behaviour, nor prevented electrocortical (Fig. 8b) or behavioural effects of nicotine (0.125 μmol)

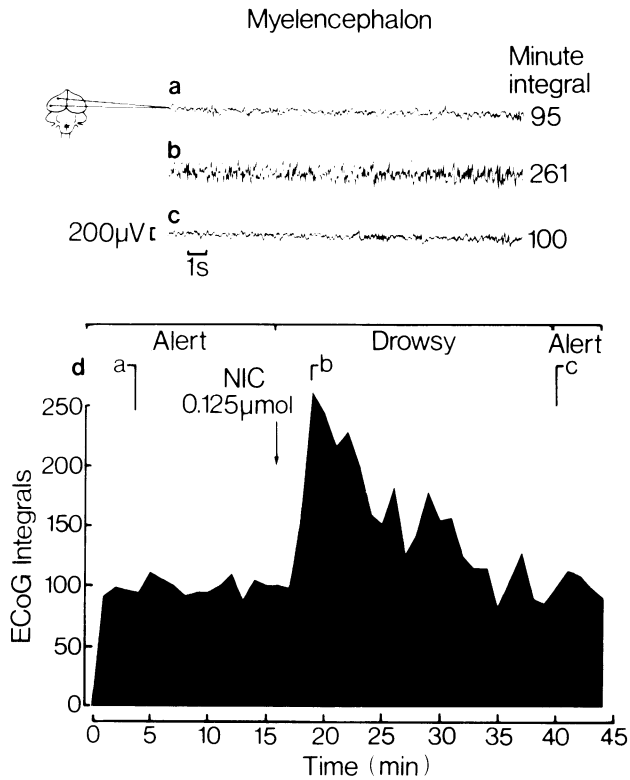

Fig. 7 Records of electrocortical activity (a to c) and histogram of integrated electrocortical activity (d) in an unanaesthetized, unrestrained 14 day chicken. Epochs corresponding to a, b and c are indicated in the histogram and integrals for the corresponding minute of electrocortical activity are given on the right of the traces. (a) Control alert electrocortical activity; (b) slow frequency (6-8 Hz), large amplitude electrocortical potentials with superimposed 'spike' potentials associated with increased electrocortical integrals (d) 3 min after nicotine (0.125 μmol) infused into the myelencephalon; (c) alert electrocortical activity on recovery from the effects of nicotine.

infused into the hypothalamus. In contrast, an identical dose of pempidine infused into the same site, in the same animal on the following day, prevented the electrocortical (Fig. 8d and e) and behavioural effects of two intrahypothalamic doses of nicotine, each of 0.125 μmol.

Discussion

Immediately after nicotine, given intraventricularly or into the brain substance, young and adult fowls squatted for about 5 min with closed eyes and beaks touching the ground. Additionally, prior to squatting and suggestive of 'irritation' by intraventricular nicotine, adult fowls scratched their heads. In non-anaesthetized cats, intraventricular physostigmine, di*iso*propylfluorophosphate or acetylcholine induced scratching of the head and other signs attributed to central irritation (Feldberg & Sherwood, 1954); intraventricular nicotine evoked violent head shaking (Armitage *et al.*, 1966). During squatting, the chickens appeared to be asleep, a state accompanied by large amplitude, slow frequency, sleep-like electrocortical potentials with superimposed 'spike' discharge. Intravenous nicotine induced similar electrocortical changes in young and adult chickens (Key & Marley, 1962), but in contrast there were marked curariform effects, the chickens lying flaccid.

There is a dearth of information concerning the electrocortical effects of nicotine given into the brain. The literature appears to consist solely of Schaeppi's results (1967, 1968), in which nicotine given into the IIIrd or IVth ventricles of gallamine-immobilized cats induced electrocortical arousal, and of our preliminary communication on

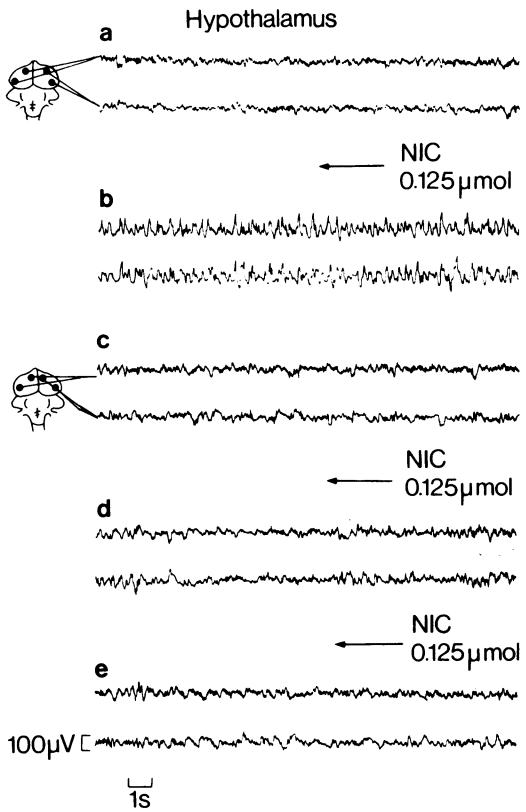

Fig. 8 Records of electrocortical activity in an unanaesthetized, unrestrained chicken tested on two consecutive days (a and b at 14 days, and c to e at 15 days). (a) Control alert electrocortical activity after prior infusion of hyoscine (0.15 μmol) into the hypothalamus; (b) hyoscine did not prevent appearance of typical large amplitude electrocortical activity after infusing nicotine (0.125 μmol) into the hypothalamus; (c) control alert electrocortical activity after infusing pempidine (0.15 μmol) in equimolar dose to hyoscine, into the hypothalamus; (d and e) pempidine prevented the effects of two intrahypothalamic infusions of nicotine each of 0.125 μmol.

the effects of nicotine in fowls (Marley & Seller, 1970a). The bulk of data relate to behavioural and electrocortical arousal elicited by nicotine given intravenously or into a carotid artery (Hall, 1970; for earlier references see Silvette, Hoff, Larson & Haag, 1962). Effects of intravenous nicotine are likely to be complicated by behavioural and electrocortical changes due to release of vasopressin and adrenal medullary catecholamines, which would intensify arousal. However, even with intravenous nicotine, behavioural and electrocortical sleep have been observed in cats and kittens but preceded by brief arousal (Marley & Key, 1963; Yamamoto & Domino, 1965). Larger doses of nicotine induced 'spike' electrocortical potentials, reminiscent of those in 'grand mal' epilepsy (Longo & Bovet, 1952; Morocutti & Sergio, 1958; Stümpf & Gogolák, 1967).

The results with young chicks suggest that nicotine acted on descending as well as ascending fibre tracts in the brain. This was because behavioural and bilateral electrocortical changes were induced by unilateral nicotine infusions even anterior and superior to the hypothalamus. Presumably, there was ipsilateral descending discharge to the thalamic nuclei, located posteriorly to the hypothalamus in chickens, with activation of ascending pathways to the contralateral hemisphere and possibly spread of impulses across the diminutive commisural tracts linking the cerebral hemispheres. In contrast, muscarine infused into similar sites evoked ipsilateral electrocortical arousal without altering behaviour, i.e. affected only ascending pathways (Marley & Seller, 1972). More posterior infusions of muscarine or nicotine, into the hypothalamus or upper brain-stem, elicited behavioural and bilateral electrocortical changes; whereas nicotine induced sleep, muscarine evoked arousal.

Tachypnoea and postural changes developed in fowls after intraventricular nicotine but not with infusions into the brain. Intraventricular muscarine elicited similar phenomena (Marley & Seller, 1972), except that latency for onset was much longer, presumably because penetration from the ventricle to sites of action was slower for muscarine compared to nicotine. Increase in respiratory rate and amplitude in cats, following intraventricular nicotine, was ascribed to its action on brain-stem structures reached from the subarachnoid space (Hall & Reit, 1966; Armitage *et al.*, 1967). Mitchell, Loeschke, Massion & Severinghaus (1963) described similar phenomena in cats following perfusion of nicotine along the ventrolateral surface of the medulla oblongata and concluded its likely site of action was a chemosensitive area between the roots of the VIIIth and XIth cranial nerves. Unfortunately, intraventricular nicotine did not elicit tachypnoea in chloralosed fowls, so the possibility of such action could not be tested, although some support for it came from findings that similar respiratory effects were obtained, albeit inconsistently, with nicotine applied externally to the brain-stem as with intraventricular injections.

The combination of tachypnoea and postural changes evoked in non-anaesthetized fowls by nicotine and muscarine resembled that induced in

fowls subjected to environmental temperatures above thermoneutrality or following drugs which elevate body temperature. However, body temperature was unaffected by nicotine, so possibly heat loss by tachypnoea offset any increased heat production. Certainly, nicotine administered centrally to various species including rat, cat and monkey, can cause hypo- or hyperthermia, depending on the method or site of injection (Hall, 1973).

In rats, performing on a Variable Ratio schedule with fluid reinforcement, nicotine (0.2 and 0.4 mg/kg s.c.) reduced bar-pressing. When 'performance was almost abolished', there was a sudden return to the rate prevailing before injection (Morrison, 1967; Morrison & Armitage, 1967). In fowls, intraventricular nicotine reduced or suppressed performance (key-pecking); reminiscent of the sudden complete recovery of performance in rats given nicotine, pecking usually resumed at a rate approximating that prior to injection.

The brief pressor effects of intraventricular nicotine in adult anaesthetized fowls proved to be mediated via the spinal cord, the vagi providing a feedback pathway necessary for rapid restoration of blood pressure to normal. Following acute bilateral vagotomy, the much prolonged pressor action of intraventricular nicotine, compared to the relatively brief prolongation after intravenous nicotine, was presumably due to excitation of brain-stem centres mediating vasoconstriction. In contrast, intraventricular nicotine usually elicited a fall in blood pressure in anaesthetized cats (Pradhan, Bhattacharya & Atkinson, 1967; Armitage & Hall, 1967b). This fall was obtained with much smaller intraventricular doses in non-anaesthetized than in anaesthetized cats (Armitage & Hall, 1967b). Like the respiratory effects, blood pressure changes induced in cats with intraventricular nicotine were ascribed to actions on brain-stem areas accessible from the subarachnoid space (Armitage *et al.*, 1967).

A number of central effects of nicotine have been attributed to the release of acetylcholine, since these were potentiated by physostigmine (Armitage *et al.*, 1966; Armitage & Hall, 1967a; Armitage *et al.*, 1967). Further support for this idea came from the finding that nicotine significantly augmented acetylcholine release from the cat cerebral cortex (Armitage, Hall & Sellers, 1969). However, in fowls, although the sleep-like behavioural and electrocortical effects of nicotine were prolonged after physostigmine, such effects were the antithesis of those after intraventricular acetylcholine and physostigmine, which evoked arousal (Marley & Seller, 1974). Consequently, acetylcholine release may not account for the behavioural and electrocortical changes induced by nicotine in fowls; intraventricular acetylcholine evoked tachypnoea as did nicotine, so acetylcholine release is feasible in this instance.

Behavioural, electrocortical and respiratory effects of nicotine in fowls were prevented or abolished by pempidine, or by serial doses of nicotine given at intervals of less than 40-60 minutes. These findings, together with the lack of antagonism by atropine or hyoscine, indicated that nicotine and not muscarine receptors were involved. Similarly, in cats, the effects of intraventricular nicotine, e.g. twitching of the ears and lowering of blood pressure, were abolished by hexamethonium perfused through the ventricle whereas hyoscine was ineffective (Hall & Reit, 1966; Armitage & Hall, 1967a). Intravenous mecamylamine or pempidine, which readily enter the central nervous system, also antagonized effects of intraventricular nicotine (Armitage *et al.*, 1966; Armitage *et al.*, 1967). Hexamethonium abolished nicotine-induced tremor (Cahen & Lynes, 1951), convulsions (Laurence & Stacey, 1952) and vomiting (Laffan & Borison, 1957).

Our present findings, and those published earlier (Marley & Seller, 1972), indicate that the fowl is a species which allows a uniquely clear differentiation between the central effects of nicotine and muscarine. To recapitulate, muscarine elicited immediate, long-lasting behavioural and electrocortical arousal, whereas after nicotine, chickens squatted for 3 to 5 min apparently asleep, the associated electrocortical pattern being that of sleep but with superimposed 'spike' activity. As these effects of nicotine waned, tachypnoea and postural changes developed; similar respiratory and postural changes developed with muscarine but, in contrast, only after a delay of 30-40 minutes. The central effects of nicotine were prevented by pempidine, but those of muscarine by hyoscine or atropine. Additionally, there was a marked difference in molar potency, nicotine being one-hundred times less active than muscarine.

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