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Effects of catecholamines infused into the brain of young chickens

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Summary

1. (−)-Noradrenaline, (−)-α-methylnoradrenaline and (−)-isoprenaline were infused into various brain regions of 12–21 day chicks. When infused into the hypothalamic area, but not the cerebral hemisphere or posterior mesencephalon, these amines produced behavioural sleep, lowered temperature and blood pressure and reduced oxygen consumption ; electrocortical sleep activity usually ensued but this was not marked and frequently dissociation between electrocortical activity and behaviour occurred. After monoamine oxidase inhibition, which prolonged the action of noradrenaline, dopamine had similar effects.

2. The effects of the catecholamines were prevented or substantially reduced by pretreatment with phenoxybenzamine given intravenously or into the hypothalamus but not by intravenous injection of propranolol. However, intrahypothalamic infusion of propranolol prevented the temperature, but not the behavioural effects of noradrenaline. The implications of this are discussed.

3. That the effects were similar but more intense, apart from electrocortical changes, and of longer duration than those seen after intravenous injection of catecholamines suggests that in young chicks these amines penetrate from the blood into the brain and elicit their effects through a localized region, presumably the hypothalamus.

Introduction

Catecholamines injected intravenously to chickens aged 1–28 days evoked behavioural and electrocortical sleep (Key & Marley, 1962 ; Dewhurst & Marley, 1965a, b) and lowered body temperature and oxygen consumption (Allen & Marley, 1967). Similar but longer lasting effects, obtained with micro-infusions of catecholamines into the hypothalamic area of 14–21 day chickens, have been briefly reported (Marley & Stephenson, 1968a, 1969) and are now given in more detail. The similarity of these effects to those produced by intravenous injection strongly suggests that the effects seen after intravenous injection are due to an action on hypothalamic nuclei since in young chickens catecholamines given intravenously penetrate to the brain, probably because of blood-brain barrier immaturity. In contrast, catecholamines given intravenously to adult fowls, in which the blood-brain barrier is mature, are either without action or elicit brief alerting (Key & Marley, 1962) whereas after intraventricular injection, so by-passing the blood-brain barrier, they produce sopor and hypothermia (Grunden & Marley, 1970). In support of blood-brain barrier immaturity, the concentration of DL-noradrenaline-7-^{3}H in whole brain

of chicks, 1 day after hatching and 2 min after intravenous injection, was 93% of the plasma concentration in contrast to 8·3% at 30 days indicating decreased penetration of noradrenaline into the brain with maturation (Spooner, Winters & Mandell, 1966).

Initial experiments were made with α-methylnoradrenaline and not noradrenaline because although their potencies were similar, the effects of α-methylnoradrenaline were more persistent (Key & Marley, 1962). The prolonged action is due to the α-methyl group which increases resistance to monoamine oxidase (Blaschko, 1952). α-Methylnoradrenaline is a false transmitter in several mammalian species but its role in the chick is uncertain since there are differences in response to α-methyl catecholamine precursors between mammalian and avian species (Juorio & Vogt, 1967). Thus, α-methyl-*m*-tyrosine, which in mammals has a reserpine-like action due to formation of metaraminol (Udenfriend & Zaltzman-Nirenberg, 1962), was without effect on catecholamines in pigeon brain (Fuxe & Ljunggren, 1965); administration of α-methyl dopa led to accumulation of α-methyl dopamine in guinea-pig brain (Schümann & Grobecker, 1965) but not in pigeon brain (Juorio & Vogt, 1967).

Methods

Animals

Rhode Island Red pullets of 80–90 g were used (age 14–21 days). They were kept in a thermostatically controlled cage maintained at 33°–34° C for the first week after hatching and for the following 2 weeks at 29°–31° C.

Construction of the micro-cannula

The micro-cannula consisted of two concentric stainless steel tubes (Accles & Pollock Ltd.); a 2 cm long 25 gauge outer tube or guide cannula which was implanted stereotactically into the brain and an inner tube (outer diameter, 0·2 mm) used for the infusion (Marley & Stephenson, 1968b). The inner cannula when not *in situ* was replaced by a stainless steel stylette.

Operative procedures

All operative procedures were performed under halothane anaesthesia (Marley & Payne, 1964). Methods for implanting cortical recording electrodes, intravenous jugular cannula (Key & Marley, 1962) and a thermistor placed subcutaneously between the scapulae (Allen & Marley, 1967) have been described previously. For recording of arterial blood pressure (1 mmHg≡1·333 mbar), a carotid artery, usually the right, was cannulated with a polyethylene cannula (PP25, Portland Plastics Ltd.) containing heparin-saline. The free end of the cannula was closed with a steel spigot and the cannula taken under the skin and brought out through a scalp incision. The arterial cannula was fixed to the skull with autopolymerizing resin at the same point where the cortical electrodes or intravenous cannula were fixed. For recording, the spigot was removed and the cannula connected through saline-filled tubing to a transducer and pen recorder.

The outer or guide cannula was implanted under aseptic conditions into a selected brain area using a Stoelting stellar stereotactic instrument (Cat. No. 51400) designed for small rodents in which the maxilla clamp, intended to fit over the rodent's

snout, was replaced by a transverse horizontal bar which could be clamped over the maxilla (upper beak) of the chick. The guide cannula was fixed to the skull with autopolymerizing resin.

Since a stereotactic atlas of the brain was not available for young chickens a stereotactic outline of the brain was constructed from the heads of four decapitated chickens (85 g, 16 days). The right frontal and parietal bones and the entire right half of the brain were removed to expose a midline sagittal view of the left side of the brain. The outline of the brain was traced with a needle held in the cannula holder, stereotactic co-ordinates were taken from the anterior-posterior and vertical scales of the instrument and transferred to graph paper. From this outline and from histological studies, co-ordinates for structures in or near the midline were obtained.

Experimental procedures

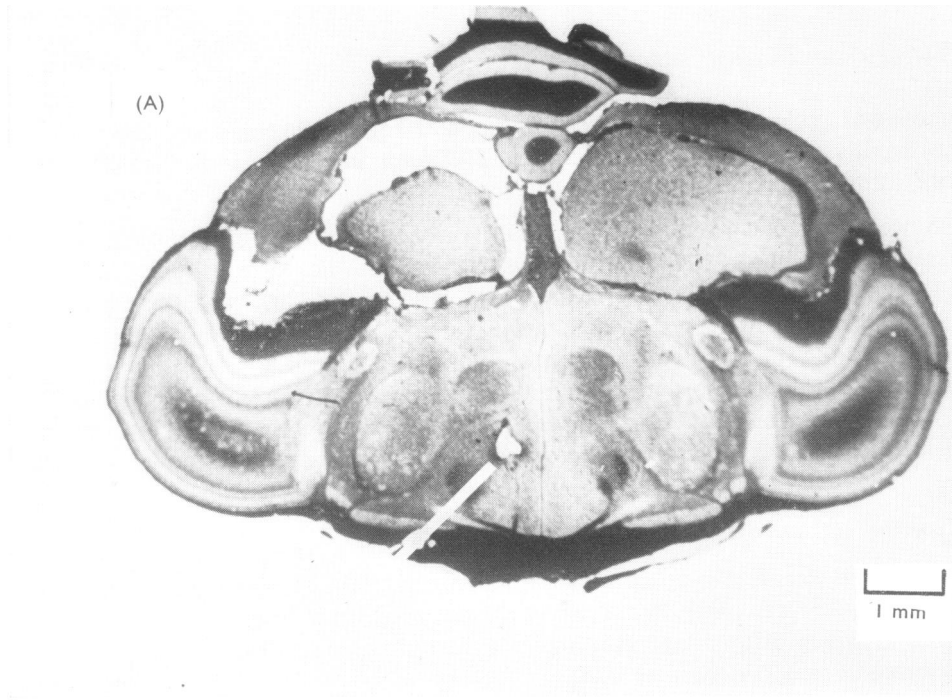
Chickens were tested at least 24 h after implantation of cannulae and electrodes when recovery was complete. Each chick was used for only one experiment except those in which drug antagonism and potentiation were studied, when experiments were made on 2 consecutive days.

About 1 h prior to the experiment the chick was placed in a soundproof, environment controlled experimental box (Stephenson, 1969) maintained at 31° C with a one-way screen and facilities for external monitoring of temperature, electrocortical activity and vocalization. Electrocortical activity was automatically integrated at one minute intervals by a modification of the method of Dewhurst & Marley (1965c), large amplitude potentials of sleep producing high integral counts and alert low voltage electrocortical patterns giving low integrals. Vocalization was recorded and quantitated by the same integrating method.

For recording of oxygen consumption, the chick was placed in a modified Richards & Collison (1928) metabolism chamber maintained in a water bath at 31° C (Allen & Marley, 1967). Falls of temperature obtained with chickens in the metabolism chamber (humidity 100%) can only be approximately compared with those obtained in the experimental box (humidity 60%) since evaporative heat loss in the box was likely to exceed that in the metabolism chamber.

Intracerebral infusions were given through a length of polyethylene tubing which passed through the roof of the box or chamber and was connected externally to a Hamilton gas-tight 1·0 ml syringe, the plunger of which was driven by a Sage slow infusion pump (No. 255-3). The tubing and syringe were filled with saline solution.

A 15 cm length of polyethylene tubing was attached to the inner portion of the micro-cannula with ' Araldite'. The micro-cannula and attached tubing were filled with a solution, in saline, of the drug to be infused. This was connected to the polyethylene tubing passing through the roof of the box. The inner cannula was then inserted into the stereotactically implanted guide cannula and infusion commenced for 4 min at a flow rate of 0·125 to 0·5 μl/minute. The cannula was left in position for the duration of the experiment unless stated otherwise. Cannulae positions were subsequently located histologically (Fig. 1A). The pattern of diffusion of 0·5 μl of a 1% solution of methylene blue determined 1 h after infusion for 4 min into the hypothalamic area was a sphere of 0·8–0·9 mm diameter (Fig. 1B).



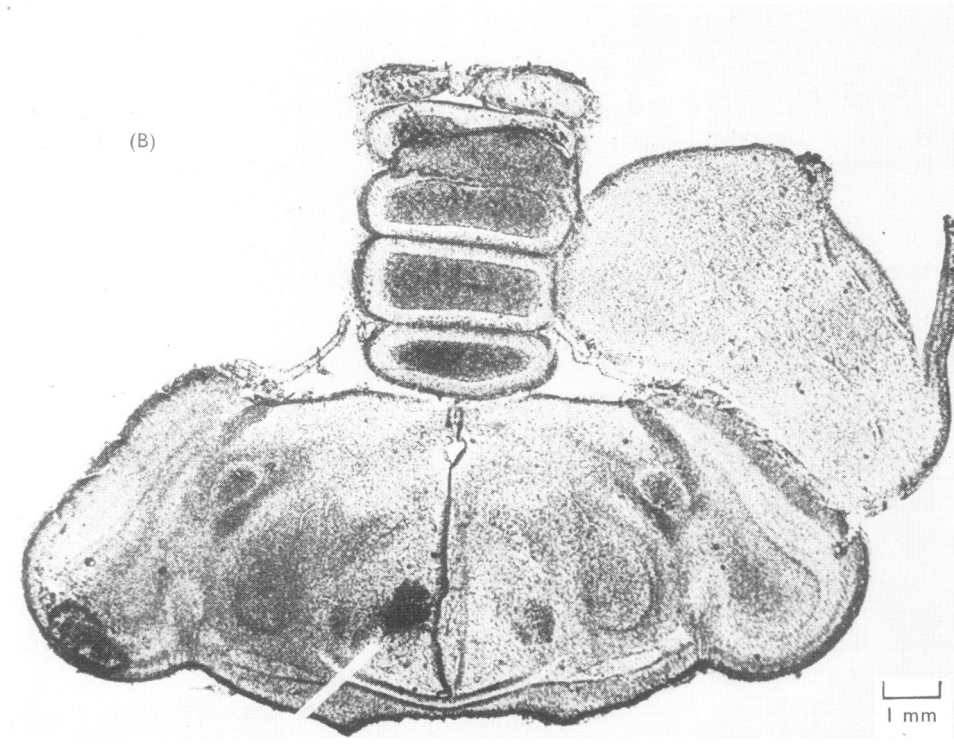


FIG. 1. A, Microphotograph of transverse section of brain to illustrate the position of the micro-cannula tip (indicated by white bar) in the posterior hypothalamic area. B, Microphotograph of an unstained transverse section of the brain to illustrate the extent of diffusion of a 1% solution of methylene blue (0·5 μl) 1 h after infusion into the posterior hypothalamic area (indicated by bar). It can be seen that the injectate could diffuse across the midline but did not diffuse out of the brain.

Drugs

Drugs used, with molecular weights in parentheses, were the hydrochlorides of (−)-α-methylnoradrenaline (220), dopamine (190), phenoxybenzamine (340) and propranolol (280); mebanazine oxalate (226) and (−)-isoprenaline sulphate (557). (−)-Noradrenaline base (169) was dissolved immediately before use in equimolar hydrochloric acid.

Results

Saline micro-infusions

Following infusions into the hypothalamus of 0·5 μl saline to six chicks, a rise in temperature of 0·5°–1·0° C was recorded in three, a fall of 0·25° C in one and no change in two. In another chick, two infusions each of 1·0 μl separated by 1 h did not alter temperature. Saline infusions lacked effect on behaviour and electrocortical activity. The solution of agonist with the lowest pH (4·8) was (−)-α-methylnoradrenaline (400 μmol/ml); infusion of 0·5 μl saline, acidified to pH 4·5 lowered temperature 0·5° C but lacked effect on behaviour and electrocortical activity.

Catecholamine micro-infusions

(−)-α-Methylnoradrenaline. α-Methylnoradrenaline was infused into the diencephalon of twenty-nine chicks. It consistently produced sleep, lowered temperature and blood pressure, diminished oxygen consumption and stopped vocalization. The effects, depending on dose and area of infusion were maximal with infusions into the hypothalamic area (Fig. 2); intensity of effect was greatest with infusions

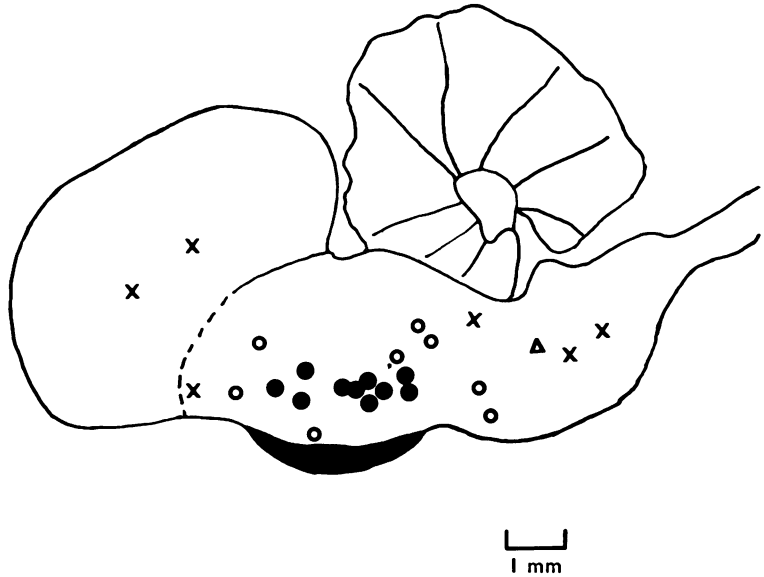


FIG. 2. Sagittal section of chick brain in midline illustrating the effects in twenty-five chicks of intracerebral infusions of (−)-α-methylnoradrenaline on temperature. ×, No effect after 0·1 or 0·2 μmol; △, fall of 1° C after 0·2 μmol; ○, fall of between 2° and 4° C after 0·1 μmol; ●, fall of greater than 4° C after 0·1 μmol or of between 2° and 4° C after 0·05 μmol.

close to the midline, but rather than suggesting an action on medial nuclei, this may represent bilateral distribution and action of the amine on hypothalamic nuclei. Infusions of 0·1–0·2 μmol into the brain stem adjacent to the trochlear nerve or into the neostriatum intermediale of the right cerebral hemisphere lacked effect.

Typical behavioural effects of α-methylnoradrenaline (0·05 μmol), infused into the posterior hypothalamic area are shown in Fig. 3. During infusion the chick's eyes closed and within 8 min it assumed a squatting position, similar to that after large intravenous doses, with the wings and beak touching the floor (Fig. 3B); the chick was not easily aroused by sensory stimuli, including handling. From 3 to 4 h after infusion the chick made occasional movements assuming at 4 h an erect sleeping position with its head lowered and the wings applied closely to the trunk (Fig. 3C), a position maintained for at least 9 hours.

Control alert electrocortical activity from the left cerebral hemisphere consisted predominantly of 8–12 Hz waves with an amplitude of 150 μV and from the right cerebral hemisphere of slower waves (4–6 Hz) with an amplitude of 100 μV (Fig. 4A). Amplitude increased and frequency slowed during control periods of drowsiness (Fig. 4B); thus, for the left cerebral hemisphere amplitude increased to 300 μV

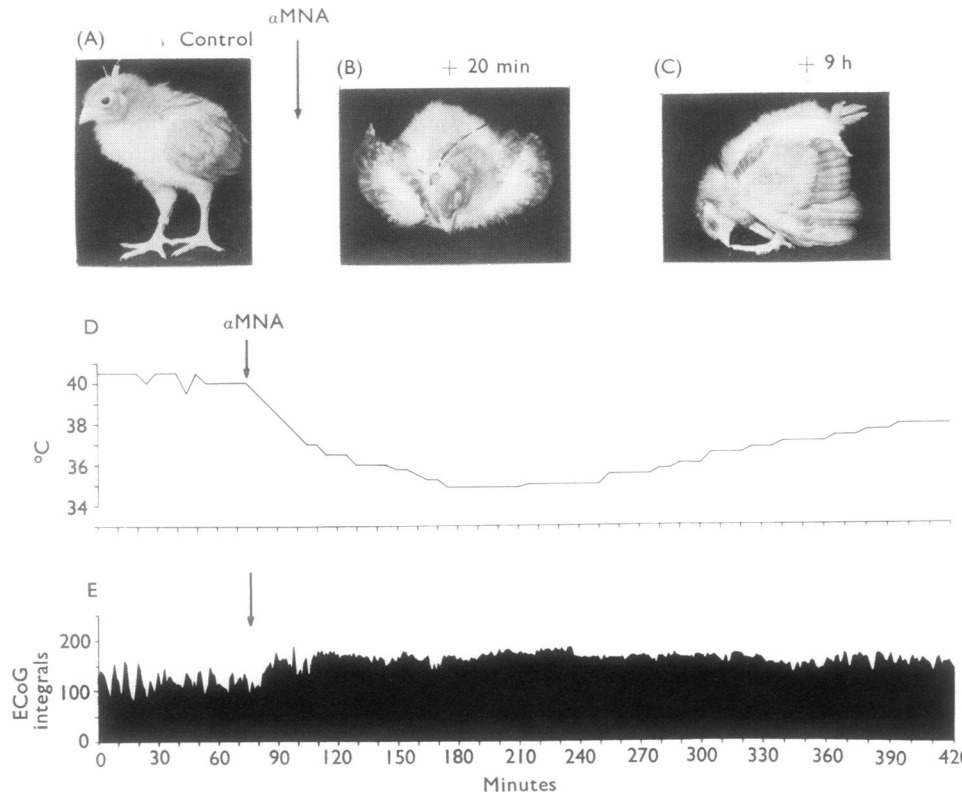


FIG. 3. Effects of an infusion of 0·05 μmol (−)-α-methylnoradrenaline (α-MNA, at arrow) into the posterior hypothalamic area on behaviour (A–C), temperature (D) and electrocortical integrals (E) of a 17 day chick (95 g). Effects on electrocortical activity are illustrated in Fig. 4. A, Control alert behaviour; B, prone sleeping posture, assumed approximately 8 min after infusion and maintained for 4 hours; C, erect sleeping posture assumed 4 h after infusion; D, lowering of temperature from 40° ±0·5° C to 34·75° C; E, increase of electrocortical integrals from left cerebral hemisphere.

with a reduction in the predominant frequency to between 4 and 6 Hz, while for the right cerebral hemisphere amplitude increased to 150 μV and the predominant frequency decreased to 2–4 Hz. After infusion of α-methylnoradrenaline, alert activity disappeared from the electrocorticogram which became similar (Fig. 4C, D) to that seen during control drowsy periods with electrocortical integrals consistently at a higher value: that is, a rise from the left cerebral hemisphere from values ranging between 75 and 155/min to between 160 and 180/min (Fig. 3E). However, large amplitude slow frequency potentials, characteristic of naturally sleeping chicks or after intravenous injections of α-methylnoradrenaline, were absent. In some chicks, electrocortical activity was dissociated from the behavioural state. Thus the chick was asleep and prostrate whilst the electrocorticogram displayed periods of electrocortical activity characteristic of a drowsy but not sleeping chick, alternating with periods of low amplitude desynchronized activity of considerably longer duration than the short (2 to 10 s) periods of paradoxical sleep activity seen during normal sleep. Concurrent with onset of sleep was a gradual fall in temperature from 40° C to 34·75° C over 2 h (Fig. 3D). Recovery of temperature paralleled that of behaviour and was complete 12 h after the infusion. Duration of response to α-methylnoradrenaline was usually dependent on its intensity and varied from between 7–12 hours.

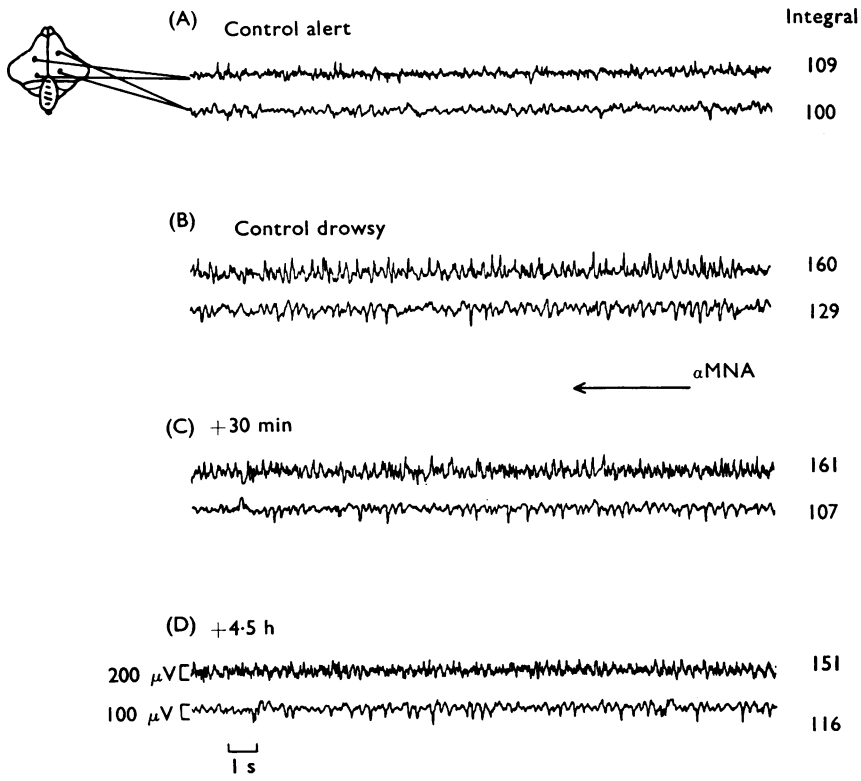


FIG. 4. Effects of an infusion of 0·05 μmol (−)-α-methylnoradrenaline (α-MNA) into the posterior hypothalamic area on electrocortical activity of a 17 day chick (95 g). Effects on behaviour, temperature and electrocortical integrals are illustrated in Fig. 3. A and B, Control alert and drowsy electrocorticograms; C and D, electrocorticograms 30 min and 4·5 h after infusion.

The effects of infusing α-methylnoradrenaline (0·01–0·1 μmol) into the hypothalamic area on oxygen consumption were determined in three chicks. Infusions of 0·01 μmol into the anterior hypothalamic area lowered temperature from 41° to 39° C and reduced oxygen consumption by 22% from (29·5 ± 1 ml/kg)/min to (23 ml/kg)/min in one and 0·1 μmol infused into the same area of a second chick, lowered temperature from 38° to 35° C and reduced oxygen consumption by 52% from (26 ml/kg)/min to (12·5 ml/kg)/min. In the third chick in which the cannula was located in the posterior hypothalamic area (Fig. 1A), 0·05 μmol reduced oxygen consumption by 21% from (19 ml/kg)/min to (15 ml/kg)/min and reduced temperature from 40·5° to 35·5° C. Depression of oxygen consumption paralleled the fall in temperature but recovery did not occur although recordings were continued for 6 h after infusion in each case.

Control mean carotid arterial blood pressure in four chicks ranged from 90 to 110 mmHg and was lowered a mean of 35 mmHg to between 55 and 70 mmHg by 0·05 μmol α-methylnoradrenaline infused into the posterior hypothalamic area (Fig. 5). The hypotensive response paralleled falls in temperature between 3° and 6° C, but recovered more rapidly.

(−)-Noradrenaline. Noradrenaline (0·05–0·075 μmol) infused into the hypothalamic area of thirty chicks lowered temperature 2·5°–6° C. Effects on behaviour, electrocortical activity, temperature and oxygen consumption were similar to those with α-methylnoradrenaline except that recovery was more rapid occurring within 3–4 h after infusion compared with at least 7 h after α-methylnoradrenaline. Thus during the control period of a representative experiment, the

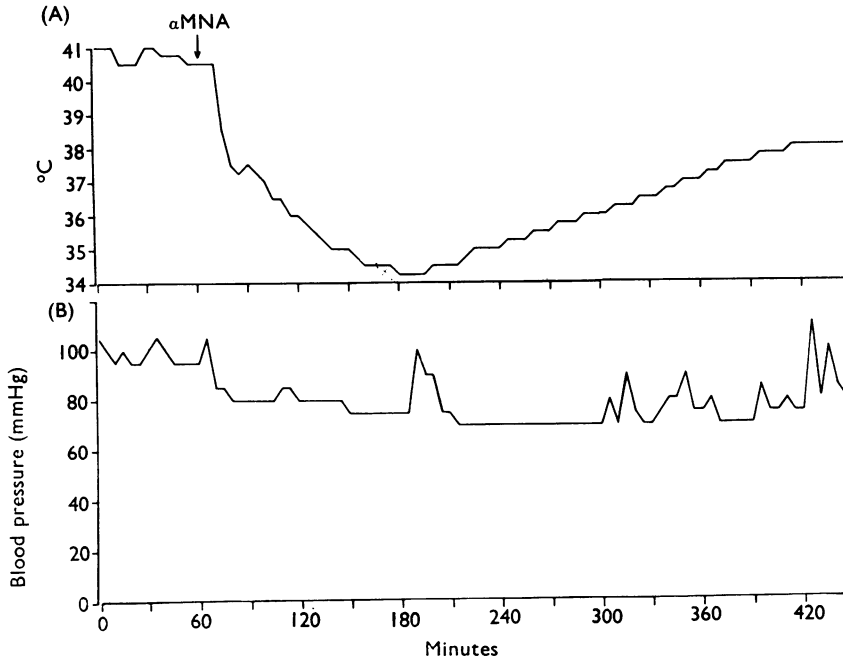


FIG. 5. Effects of an infusion of 0·05 μmol (−)-α-methylnoradrenaline (α-MNA, at arrow) into the posterior hypothalamus of a 15 day chick (85 g). A, Lowering of temperature from 40·75 ± 0·25° C to 34·25° C with partial recovery after 6 hours; B, lowering of blood pressure from 100 ± 10 mmHg to 70 mmHg.

chick displayed alternate alert and drowsy behaviour with electrocortical integrals ranging from 70 to 210/min (Fig. 6B). Electrocortical activity varied from high amplitude, slow wave activity (250 μV ; less than 2 Hz) to low amplitude fast frequency activity (30 μV ; 20–30 Hz). Within 10 min of commencing an infusion of 0·05 μmol noradrenaline, the chick fell asleep in a squatting position, similar to that seen after α-methylnoradrenaline. Electrocortical integrals from both cerebral hemispheres increased over 1 h and were maintained at about 200/min and the electrocorticogram consisted almost entirely of higher amplitude activity. The chick remained squatting for approximately 2 h after which it occasionally moved around the box only to resume squatting. From 2·5 to 3 h after infusion the chick was erect for part of the time but still asleep and from 3 to 4 h it exhibited alert periods interspersed with those of sleep when electrocortical integrals ranged from 70 to 340/min. The extreme sleeping position with wings and beak touching the ground observed after α-methylnoradrenaline did not develop. Temperature fell from 41° to 35° C, returning to 41·5° C within 4 h of the infusion (Fig. 6A).

Fig. 7 illustrates depression of oxygen consumption and fall in temperature produced by noradrenaline, 0·05 μmol. Control mean oxygen consumption was (33 $\pm$ 2 ml/kg)/minute. Recording was not resumed until 30 min after infusion, because of the period required for re-equilibration of the chamber, by which time oxygen consumption had fallen 24% to (25 ml/kg)/min ; temperature declined over the same period from 40·75° to 38·25° C. Recovery of behaviour and oxygen consumption occurred within 2·5–3 h of the infusion and temperature after 4 hours.

(−)-Isoprenaline. Infused into the hypothalamic area, isoprenaline produced effects similar to those of α-methylnoradrenaline ; its potency was approximately half that of α-methylnoradrenaline.

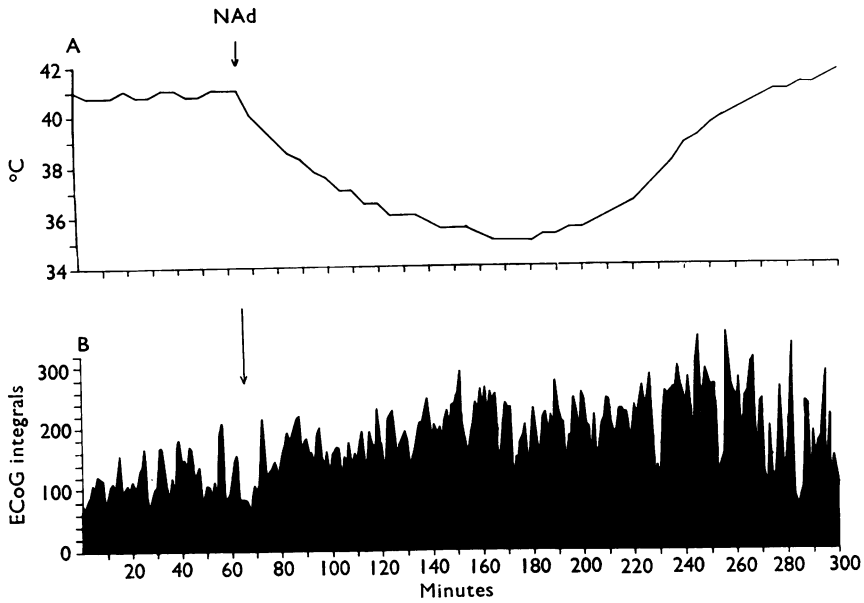


FIG. 6. Effects of an infusion of 0·05 μmol (−)-noradrenaline (NAD, at arrow) into the posterior hypothalamic area on temperature and electrocortical integrals of a 14 day chick (90 g). A, Lowering of temperature from 41° to 35° C with recovery after 4 hours ; B, increase in electrocortical integrals with gradual recovery and diminution after 3–4 hours.

In five chickens, isoprenaline (0·05–0·1 μmol) infused into the region of the posterior hypothalamic nucleus and the lateral hypothalamic nucleus lowered temperature 1·75°–2·75° C; in three other chicks in which it was infused slightly more posteriorly, 0·05 μmol lowered temperature 3·75°–4·75° C. Duration of temperature fall was 5–6 h (Fig. 8) and recovery was protracted as after α-methylnoradrenaline but the behavioural effects were less. Sleep and squatting did occur, but often not until 30 min after infusion. Increase in electrocortical integrals ranged from 15 to 100%, the smaller increases generally occurring in chicks drowsy during the control period.

Dopamine. Dopamine was infused into the posterior hypothalamic area of four chicks. Three received 0·1 μmol followed after 1 h by a further infusion of 0·2 μmol; one received 0·05 μmol followed 1 h later by 0·2 μmol. In each case, the first infuson was without effect on temperature but in two chicks temperature gradually rose 1° C after the second infusion (Fig. 9A). In the two remaining chicks, the second infusion produced a fall in temperature of 1° C, which recovered within 30–40 min followed by a rise in temperature of 0·5° C. Electrocortical activity, electrocortical integrals (Fig. 9A) and behaviour were unaltered.

Antagonism and potentiation

Experiments on antagonism or the effects of pretreatment with inhibitors of monoamine oxidase were performed in each chicken on 2 consecutive days. To

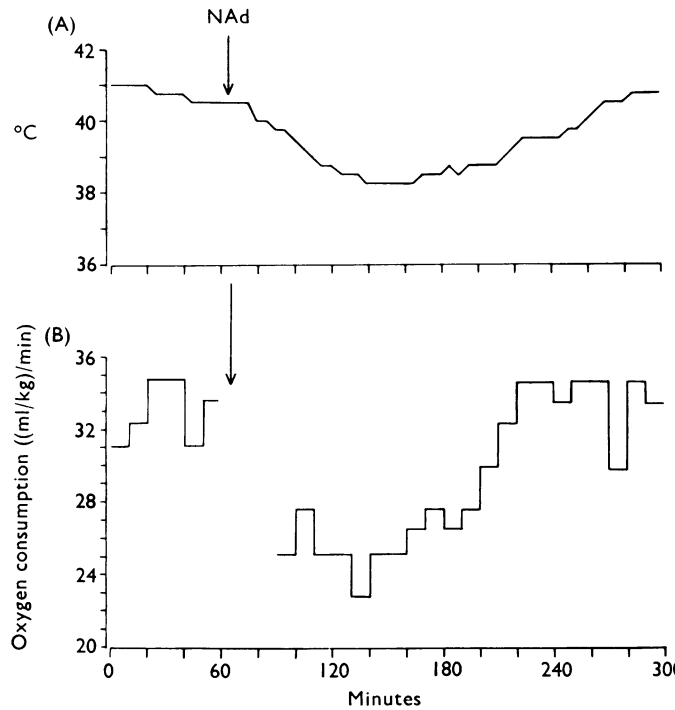


FIG. 7. Effects of an infusion of 0·05 μmol (−)-noradrenaline (NAd, at arrow) into the posterior hypothalamic area on temperature and oxygen consumption of a 16 day chick (85 g). Recording of oxygen consumption ceased for 30 min after infusion until thermal equilibrium in the oxygen consumption chamber was re-established. A, Lowering of temperature from 40·5° ±0·25° C to 38·25° C; B, reduction of oxygen consumption from (33±2 ml/kg)/min to (25 ml/kg)/minute.

ascertain reproducibility of drug effects in the absence of potentiation or antagonism, five chicks each received an infusion of 0·5 μmol α-methylnoradrenaline on 2 consecutive days. On the first, falls of temperature ranging from 2° C to 4·5° C were obtained. On the second, the fall of temperature in each chick was within 0·5° C of that obtained the day previous and of the same duration. The behavioural effects were of similar intensity and duration on each day.

Effect of monoamine oxidase inhibition

Noradrenaline. In two chicks pretreated with mebanazine (10 μmol/100 g intravenously 18 h and 30 min before infusion of noradrenaline) which itself did not influence behaviour, electrocortical activity or temperature, noradrenaline 0·05 μmol and 0·075 μmol lowered temperature the same amount as in control experiments (6° C and 5° C respectively) performed 24 h previously, but the duration of the fall was increased to 8–9 h (compare Fig. 10B with Fig. 10A). Behavioural and electrocortical effects were similarly prolonged and recovery was protracted as after α-methylnoradrenaline. In both experiments the inner cannula was removed 4 h after infusion, the time of complete recovery in control experiments.

Dopamine. Four chicks which 24 h previously had received infusions of dopamine (q.v.) were pretreated with mebanazine (10 μmol/100 g intravenously) 18 h and 30 min before a further infusion of dopamine. Dopamine, 0·1 μmol now produced sleep within 10 min of infusion, concurrent with increases in electro-

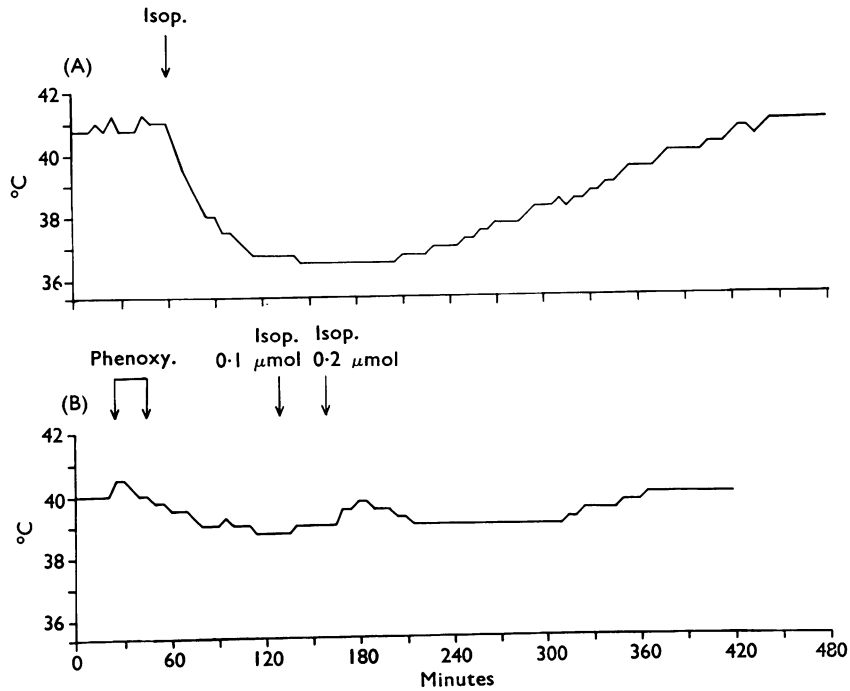


FIG. 8. Graphs of temperature from a 16 to 17 day chick (85 g). A, Fall in temperature with recovery in 7 h produced by an infusion of 0·1 μmol (−)-isoprenaline (Isop, at arrow) into the posterior hypothalamic area; B, same chick 24 h later given phenoxybenzamine (Phenoxy, 10 μmol/100 g intravenously at arrow). Between 1·5 and 2 h later infusions of 0·1 and 0·2 μmol (−)-isoprenaline (Isop, at arrows) were without effect on temperature.

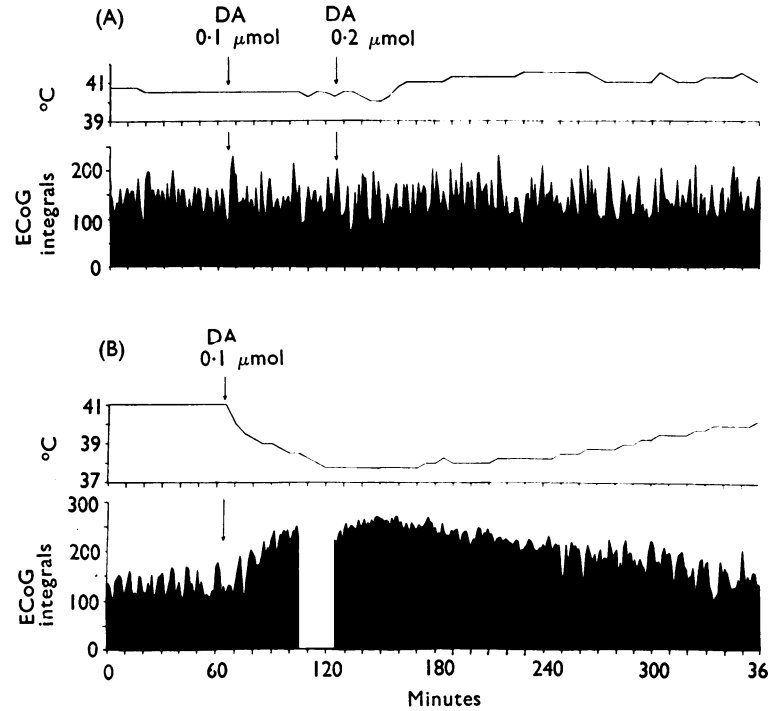


FIG. 9. Graphs of temperature and histograms of electrocortical integrals from a 13 to 14 day chick (85 g). A, Lack of effect of two infusions of dopamine (DA, 0·1 and 0·2 μmol) into the posterior hypothalamic area on electrocortical integrals. There was a rise in temperature of 1° C after the second infusion. B, Same chick 24 h later after pretreatment with mebanazine (10 μmol/100 g) at 18 h and 30 min before infusion of dopamine. Dopamine (DA, 0·1 μmol) now lowered temperature from 41° C to 38° C and increased electrocortical integrals. (The gap in recording of electrocortical integrals from 105 to 125 min after dopamine was to allow photography of the behavioural changes.)

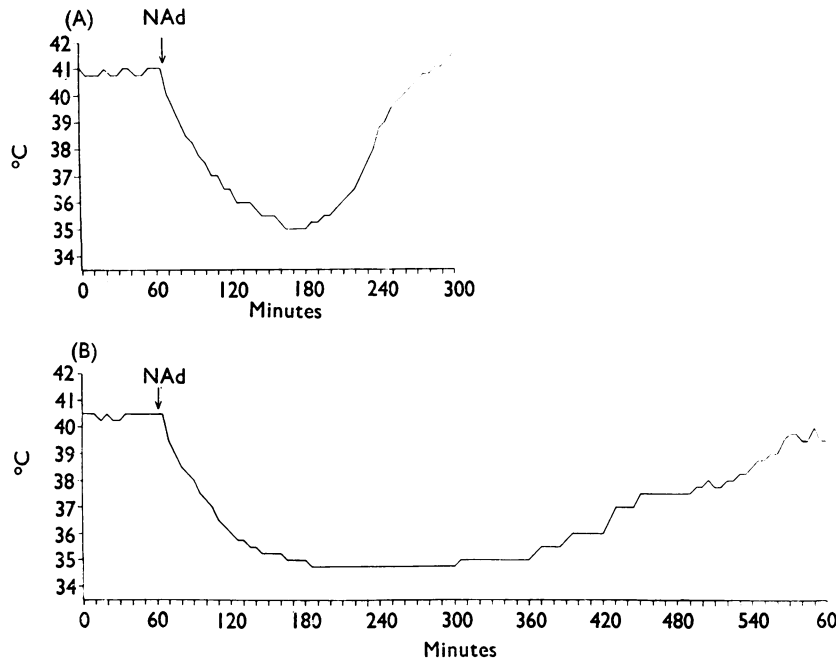


FIG. 10. Graphs of temperature from a 14 to 15 day chick (90 g). A, Fall in temperature with recovery in 4 h produced by an infusion of 0·05 μmol (−)-noradrenaline (NAd, at arrow) into the hypothalamus; B, same chicken 24 h later given mebanazine (10 μmol/100 g intravenously) 18 h and 30 min previously showing prolonged fall in temperature with recovery in 9 h produced by a similar infusion of (−)-noradrenaline.

cortical integrals of 55–100%; temperature was lowered between 3° and 5° C (Fig. 9B). The chick recovered within 5–6 hours.

Effect of an antagonist at peripheral sympathetic α-adrenoceptors

(−)-α-Methylnoradrenaline. Intravenous injection of phenoxybenzamine prevented the behavioural, electrocortical and temperature effects of α-methylnoradrenaline infused into the hypothalamus of four chicks. In a representative experiment, a control infusion of α-methylnoradrenaline, 0·05 μmol, lowered temperature from 40·25 ± 0·25° to 37° C with recovery in 7 h and produced behavioural sleep lasting 6 hours. Electrocortical integrals, which before infusion had fluctuated between 80 and 180/min, were now constantly above 180/min. On the following day, phenoxybenzamine, 10 μmol/100 g intravenously lowered temperature from 40·5° to 38·75° C and produced behavioural alerting; electrocortical integrals ranged from 160 to 180/minute. Two infusions of α-methylnoradrenaline, each of 0·1 μmol, given 2 and 3 h after phenoxybenzamine lacked effect on temperature, electrocortical activity and behaviour.

(−)-Noradrenaline: (a) Intravenous phenoxybenzamine. Phenoxybenzamine, 10 μmol/100 g, prevented the effects of noradrenaline infusion in only three out of seven chicks; this contrasts with consistent antagonism of α-methylnoradrenaline and isoprenaline. Since intravenous phenoxybenzamine lowered temperature to such an extent that several experiments had to be abandoned, it was infused instead into the hypothalamus prior to noradrenaline.

(b) Intrahypothalamic phenoxybenzamine. Phenoxybenzamine infused into the hypothalamic area prevented or substantially reduced the effects of noradrenaline infused 1·5 to 2 h later in eight out of ten chicks; phenoxybenzamine itself lacked effect on behaviour and temperature. In a representative experiment, a control infusion of noradrenaline, 0·025 μmol, lowered temperature from 40·75° to 37·25° C with recovery after 3 h (Fig. 11A) and produced behavioural sleep lasting 2·5–3 h; electrocortical activity was not recorded. Twenty-four hours later, although temperature rose slightly from 40·25° to 40·75° C after an infusion of phenoxybenzamine, 0·1 μmol (2 μl in 16 min; Fig. 11B), behaviour was unaffected. Infusions of noradrenaline, 0·025 μmol and 0·05 μmol, approximately 2 and 3 h respectively after phenoxybenzamine, now lacked effect on temperature (Fig. 11B) and behaviour.

(−)-Isoprenaline. Response to isoprenaline was prevented by intravenous injection of phenoxybenzamine in four chicks after control hypothermic and soporific effects of isoprenaline had been obtained the day previous. In a representative experiment, a control infusion of isoprenaline, 0·1 μmol, lowered temperature 4·75° C with recovery after 7 h and produced behavioural sleep lasting 6 hours. Electrocortical integrals from the left cerebral hemisphere increased from between 120 and 180/min to between 200 and 280/min while those from the right cerebral hemisphere increased from between 90 and 130/min to 190/min. Twenty-four hours later, phenoxybenzamine, 10 μmol/100 g intravenously, produced behavioural and electrocortical alerting with a reduction in electrocortical integrals from between 120 and 220/min from both cerebral hemispheres to between 80 and 140/min; temperature fell 1° C to 40° C. An infusion of isoprenaline, 0·2 μmol, 90 min later lowered temperature a further 0·5° C to 39·5° C but a second infusion of 0·2 μmol 1 h after the first infusion, raised temperature over the ensuing 100 min to

41° C. Electrocortical integrals increased to between 120 and 160/min but there were no behavioural changes. Antagonism of the hypothermic effects of isoprenaline by phenoxybenzamine in another chick is illustrated in Fig. 8.

Effect of an antagonist at peripheral sympathetic β-adrenoceptors

(−)-Noradrenaline: (a) Intravenous propranolol. Propranolol did not prevent hypothermic and behavioural effects of hypothalamic infusions of noradrenaline in four chicks. In a representative experiment, a control infusion of noradrenaline, 0·05 μmol, lowered temperature 2·5° C and produced behavioural and electrocortical sleep, effects lasting 3 hours. On the following day, noradrenaline, 0·05 μmol infused 30 min after intravenous injection of propranolol, 0·5 μmol/100 g lowered temperature 2° C with recovery in 3 h; similarly, behavioural and electrocortical effects were not prevented.

(b) Intrahypothalamic propranolol. Propranolol infused into the hypothalamic area prevented or substantially reduced the effects of noradrenaline infused 1·5 to 2 h later in four chicks; propranolol itself did not affect temperature or behaviour. In a representative experiment, noradrenaline, 0·025 μmol, lowered temperature 2·5° C from 41° to 38·5° C with recovery in 2·5 h and produced behavioural sleep lasting 2 hours. Twenty-four hours later, infusion of noradrenaline, 0·025 μmol, 2 h after infusion of propranolol, 0·1 μmol (2 μl in 16 min) lowered temperature 0·75° C only. In contrast, the behavioural effects were as intense as those observed with noradrenaline alone.

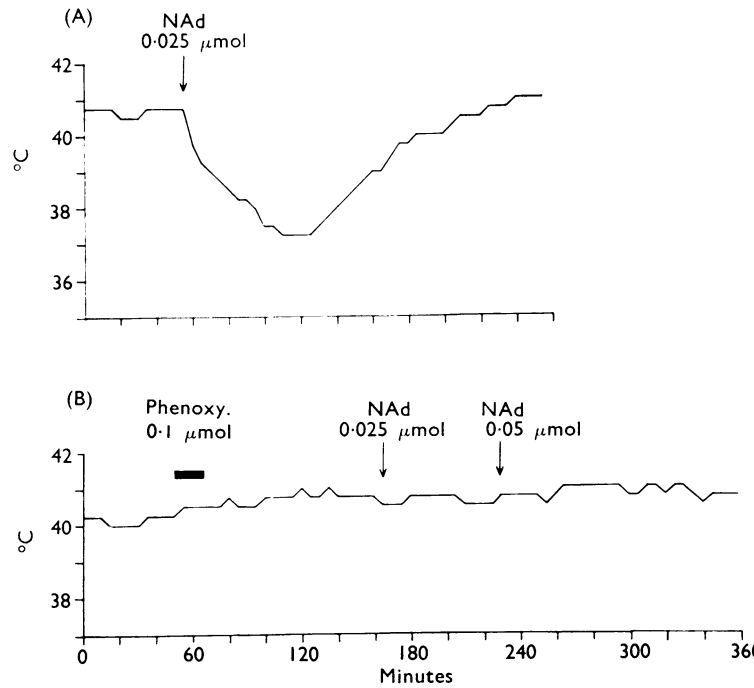


FIG. 11. Graphs of temperature from a 12–13 day chick (80 g). A, Fall in temperature of 3·5° C with recovery in 3 h produced by an infusion of 0·025 μmol (−)-noradrenaline (NAd, at arrow) into the hypothalamic area. B, Same chick 24 h later given an infusion of phenoxybenzamine (0·1 μmol, at bar) into the hypothalamus. Approximately 2 and 3 h later infusions of 0·025 and 0·05 μmol (−)-noradrenaline (NAd, at arrows) lacked effect on temperature.

(−)-Isoprenaline. Two chickens each received propranolol, 1·0 μmol/100 g intravenously, 30 min before infusion of isoprenaline, 0·1 μmol. In the control experiments, this dose of isoprenaline produced behavioural and electrocortical sleep and lowered temperature 1·7° to 2·2° C respectively, effects persisting for 6 hours. After propranolol, isoprenaline lowered temperature 3° and 2° C respectively and evoked behavioural and electrocortical sleep of similar duration to that obtained in the control experiments.

Discussion

Infusion of catecholamines into the ventral diencephalon or hypothalamus produced sleep with falls of temperature, blood pressure and oxygen consumption. Behavioural sleep and fall in temperature were more intense and prolonged whereas the electrocortical effects were less marked than those after intravenous injection. Although these effects were maximal and of most rapid onset with infusions into or medial to the lateral hypothalamus, they need not result from action solely on this nucleus since this volume of infusate (0·5–2·0 μl in 4 min) encompasses several ipsilateral and possibly contralateral nuclei in the diminutive chick brain (see **Methods**). Indeed, it is unlikely that such diverse effects would be elicited by an action on a single nucleus.

The amounts required to produce these effects, although much smaller (1/100 to 1/40) than those needed to evoke similar changes after intravenous injection, may be greater than that in the hypothalamus. The concentration of noradrenaline determined by extraction from the hypothalamus is no guide since that attained at synapses would probably exceed the overall hypothalamic concentration. In addition, some of the noradrenaline infused would be taken up by neurones or removed by capillaries during diffusion to synapses.

Various terms have been used to describe the central depressant effects of catecholamines in different species. These include, decreased locomotor activity (Fischer & Amalfara, 1962 ; Grunden & Katzung, 1964 ; Grossman, 1968 ; Grunden, 1969) ; sedation (Breggin, 1965 ; Findlay & Robertshaw, 1967), stupor or partial anaesthesia (Palmer, 1959 ; Breggin, 1965), catalepsy (Traczyk, 1964), analgesia (Ivy, Goetzl, Harris & Burrill, 1944) and anaesthesia (Leimdorfer & Metzner, 1949 ; Feldberg & Sherwood, 1954 ; Reitter, 1957). Catecholamines given intravenously or by cerebral micro-infusion in young chicks or by intraventricular injection in adults produced effects indistinguishable from normal sleep (Key & Marley, 1962 ; Dewhurst & Marley, 1965a & b ; Marley & Stephenson, 1968a, 1969 ; Grunden & Marley, 1970). In cats, small doses of adrenaline (5–10 μg) micro-injected into the anterior, posterior, lateral or medial hypothalamic areas produced a drowsy almost motionless state, whereas larger doses (25–50 μg) produced " compulsive " sleep which was at most briefly interrupted by sensory stimuli (Myers, 1964).

In view of the striking slow wave electrocortical activity following small intravenous doses of catecholamines, its intensification by larger amounts (Dewhurst & Marley, 1965a) and of the known integrative action of the hypothalamus, the moderate electrocortical changes were surprising. Possibly brain mechanisms producing large amplitude slow wave electrocortical sleep lay outside the hypothalamus, or this was a type of electrocortical activity associated with extremely deep sleep. In dogs and cats, intracisternal injections of adrenaline in doses which produced

" anaesthesia " lacked significant electrocortical effects (Leimdorfer, Arana & Hack, 1947; Leimdorfer & Metzner, 1949; Leimdorfer, 1950); indeed, there may even be dissociation between behaviour and electrocortical activity after intraventricular injection of adrenaline (Rothballer, 1959).

Intraventricular injections of adrenaline or noradrenaline lowered body temperature in cats (Feldberg & Myers, 1964) as did smaller amounts injected into the anterior hypothalamus (Feldberg & Myers, 1965). In chicks, infusions of catecholamines into the diencephalon lowered body temperature more markedly than in other species. Two explanations can be advanced for this. First, because of poor thermal insulation and high heat loss chicks are particularly sensitive to decreases in heat production; at 29·5° C, heat loss from a 14 day chick is (7·85 calories/h)/g, compared with (2·85 calories/h)/g for a 52 week old chick (Barott & Pringle, 1946). Second, because of fluctuations in body temperature ($\pm$0·5° C) under control conditions, doses of catecholamines were given to ensure temperature falls outside this range. Lesion studies suggest the existence of a heat loss centre in the anterior hypothalamus and a heat production centre in the posterior lateral hypothalamus of adult fowls (Kanematsu, Kii, Sonoda & Kato, 1967) similar to those postulated for mammals (Ström, 1960).

A central action leading to a lowering of body temperature by increased heat loss and/or decreased heat production, involves peripheral factors. Thus lowering of blood pressure after micro-infusion of α-methylnoradrenaline into the hypothalamus may be due to vasodilatation so increasing heat loss. This contrasts with the marked hypertension and vasoconstriction after its intravenous injection, also associated with hypothermia. Slight reduction in metabolic rate might be expected secondary to sleep and muscular relaxation induced by catecholamines. However, muscular relaxation induced by intravenous infusion of *d*-tubocurarine ((0·1 mg/kg)/min) did not significantly affect oxygen consumption until respiration was seriously impaired (Marley & Stephenson, unpublished results). Nor was there any reason for supposing that altered secretion of pituitary hormones played any part in the temperature fall, for although repeated micro-injections of adrenaline into the mammillary body or ventromedian nucleus of the rabbit hypothalamus inhibited release of ^{131}I from the thyroid gland (Harrison, 1961), even if inhibition had been instantaneous there would still be sufficient tissue or circulating thyroxine for heat production.

Unexpectedly, dopamine lacked effects in untreated chicks since small intravenous doses (0·1–0·3 mg/kg) have central depressant actions (Key & Marley, 1962). Spooner & Winters (1965) reported initial excitation after large intraperitoneal doses of dopamine (75–100 mg/kg) but after 30 min sleep ensued attributed to noradrenaline formation. In our chicks, even after a second micro-infusion of dopamine, behavioural depression and hypothermia did not develop. Rapid synthesis of noradrenaline from dopamine occurs in the hypothalamus of rats where a maximum concentration of ^{3}H-noradrenaline is reached 30 min after intraventricular injection of ^{3}H-dopamine (Glowinski & Iversen, 1966). Since synthesis takes place in neurones, the lack of depressant action due to noradrenaline in such circumstances might suggest that after synthesis it was not released by the neurones but taken up and retained by the storage vesicles particularly if they were only partly filled (Aghajanian & Bloom, 1966).

After pretreatment with mebanazine, dopamine produced effects similar to those of other catecholamines. Since these effects were of rapid onset they could at least partly be attributed to dopamine. Dopamine lacks a hydroxyl group on the β-carbon atom and consequently is more rapidly deaminated than adrenaline or noradrenaline (Blaschko, Richter & Schlossman, 1937). Benzylhydrazines are potent inhibitors of dopamine β-hydroxylase *in vitro* and *in vivo* (Creveling, van der Schoot & Udenfriend, 1962; Kuntzman, Costa, Creveling, Hirsch & Brodie, 1962) so the soporific effects could be due to a prolonged action of dopamine although the inhibitory activity of mebanazine (α-methyl benzylhydrazine) on dopamine β-hydroxylase is not known. Pretreatment with a monoamine oxidase inhibitor increases the concentration of noradrenaline in the chicken brain (Pscheidt & Himwich, 1965) possibly filling storage granules with noradrenaline so that excess noradrenaline, newly synthesized from dopamine, overflows from the neurone and is released as free noradrenaline instead of the deaminated metabolite.

Response to noradrenaline was prolonged by pretreatment with mebanazine. Hypothermia in rats produced by intracerebral injection of noradrenaline is potentiated by nialamide (Schmidt & Fähse, 1964). Disappearance from the rat brain of ^{3}H-noradrenaline taken up after intraventricular injection, occurs in two phases with half-lives of 3–4 and 17–18 h respectively (Glowinski, Kopin & Axelrod, 1965). Peripheral tissues deal similarly with noradrenaline but after monoamine oxidase inhibition, the slower release phase occurs over a longer period (Axelrod, Hertting & Patrick, 1961). A delayed release or an overflow of free noradrenaline could explain its prolonged central depressant action after mebanazine. This implies that initial uptake of amine into the cell is important for potentiation of response since monoamine oxidase activity is localized mainly in neuronal mitochondria (Blaschko, 1963; Oswald & Strittmatter, 1963). However, isoprenaline had effects similar to those of noradrenaline but in contrast is taken up in negligible quantities by rat tissues *in vivo* (Hertting, 1964) and by rat brain cortex slices *in vitro* (Ross & Renyi, 1966).

Qualitatively similar effects were obtained with infusion into the hypothalamus of noradrenaline, an agonist at α-adrenoceptors for catecholamines, and isoprenaline, an agonist at β-adrenoceptors. That the effects were mediated by α-adrenoceptors was suggested by experiments in which antagonists were given intravenously since the effects were prevented by phenoxybenzamine, but not by propranolol, in the dose used. Whereas intravenous phenoxybenzamine consistently prevented the actions of α-methylnoradrenaline and isoprenaline, antagonism of noradrenaline was less readily obtained (three out of seven experiments), possibly because of the high efficacy of noradrenaline for α-adrenoceptors and difficulties in obtaining complete α-adrenoceptor blockade in the fowl (Harvey & Nickerson, 1951; Dewhurst & Marley, 1965a; Marley & Stephenson, personal observations). A further disadvantage of intravenous phenoxybenzamine was that it reduced body temperature. This was probably due to peripheral vasodilatation and associated heat loss. Response to noradrenaline was therefore studied after intrahypothalamic infusion of antagonists. Unexpectedly, propranolol as well as phenoxybenzamine micro-infused into the hypothalamus prevented the effects on temperature of noradrenaline given two hours later. In contrast, phenoxybenzamine, but not propranolol prevented the behavioural effects. There are a number of explanations for these results. First, blockade produced by phenoxybenzamine and/or propranolol was not selective.

Second, the receptors are different from peripheral α- and β-adrenoceptors. Third, the action of catecholamines on temperature is sensitive to both α- and β-adrenoceptor blockade. Indeed, the situation may be analogous to that in rat adipose tissue during catecholamine induced lipolysis where phentolamine produces a non-competitive block and Kö-592, an antagonist at β-adrenoceptors, produces competitive block (Stock & Westermann, 1966). Whereas phentolamine inhibited the action of already formed adenosine-3′,5′-monophosphate (3′,5′-AMP), which mediates catecholamine induced lipolysis, Kö-592 inhibited 3′,5′-AMP formation. This explanation assumes involvement of 3′,5′-AMP in the action of catecholamines on temperature, a possibility that is being tested.

Assuming that blockade produced by propranolol infusion into the hypothalamus was selective and since propranolol readily enters the brain (Masuoka & Hansson, 1967), the most likely explanation for lack of antagonism after its intravenous injection was insufficiency of dose. However, the dose was at least 10 times that required to prevent cardiovascular actions of isoprenaline in the fowl ; larger intravenous doses caused behavioural excitement. It was concluded that receptors similar to peripheral α-adrenoceptors mediate behavioural effects of catecholamines infused into the hypothalamus but that β-adrenoceptors, or a catecholamine receptor with α and β characteristics may be involved in the temperature response. Further experiments are necessary to elucidate the nature of these receptors.

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REFERENCES

AGHAJANIAN, G. K. & BLOOM, F. E. (1966). Electron-microscopic autoradiography of rat hypothalamus after intraventricular H^3-norepinephrine. *Science, N.Y.*, **153**, 308–310.

ALLEN, D. J. & MARLEY, E. (1967). Effect of sympathomimetic and allied amines on temperature and oxygen consumption in chickens. *Br. J. Pharmac. Chemother.*, **31**, 290–312.

AXELROD, J., HERTTING, G. & PATRICK, R. W. (1961). Inhibition of H^3-norepinephrine release by monoamine oxidase inhibitors. *J. Pharmac. exp. Ther.*, **134**, 325–328.

BAROTT, H. G. & PRINGLE, E. M. (1946). Energy and gaseous metabolism of the chicken from hatch to maturity as affected by temperature. *J. Nutrit.*, **37**, 35–50.

BLASCHKO, H. (1952). Amine oxidase and amine metabolism. *Pharmac. Rev.*, **4**, 415–458.

BLASCHKO, H. (1963). Amine oxidase. In: *The Enzymes*, ed. Boyer, P. D., Lardy, H. A. & Myrback, K., vol. **8**, pp. 337–351. New York, Academic Press Inc.

BLASCHKO, H., RICHTER, D. & SCHLOSSMAN, H. (1937). The oxidation of adrenaline and other amines. *Biochem. J.*, **31**, 2187–2196.

BREGGIN, P. R. (1965). Sedative-like effect of epinephrine. *Archs gen. Psychiat.*, **12**, 255–259.

CREVELING, C. R., VAN DER SCHOOT, J. B. & UDENFRIEND, S. (1962). Phenethylamine isosteres as inhibitors of dopamine β-oxidase. *Biochem. Biophys. Res. Comm.*, **8**, 215–219.

DEWHURST, W. G. & MARLEY, E. (1965a). The effects of α-methyl derivatives of noradrenaline, phenethylamine and tryptamine on the central nervous system of the chicken. *Br. J. Pharmac. Chemother.*, **25**, 682–704.

DEWHURST, W. G. & MARLEY, E. (1965b). Action of sympathomimetic and allied amines on the central nervous system of the chicken. *Br. J. Pharmac. Chemother.*, **25**, 705–727.

DEWHURST, W. G. & MARLEY, E. (1965c). Methods for quantifying behaviour and cerebral electrical activity and the effects of drugs under controlled conditions. *Br. J. Pharmac. Chemother.*, **25**, 671–681.

FELDBERG, W. & MYERS, R. D. (1964). Effects on temperature of amines injected into the cerebral ventricles. A new concept of temperature regulation. *J. Physiol., Lond.*, **173**, 226–237.

FELDBERG, W. & MYERS, R. D. (1965). Changes in temperature produced by micro-injections of amines into the anterior hypothalamus of cats. *J. Physiol., Lond.*, **177**, 239–245.

FELDBERG, W. & SHERWOOD, S. L. (1954). Injections of drugs into the lateral ventricles of the cat. *J. Physiol., Lond.*, **123**, 148–167.

FINDLAY, J. D. & ROBERTSHAW, D. (1967). The mechanism of body temperature changes induced by intraventricular injections of adrenaline, noradrenaline and 5-hydroxytryptamine in the ox (*Bos taurus*). *J. Physiol., Lond.*, **189**, 329–336.

FISCHER, E. & AMALFARA, M. L. (1962). Effect of catecholamines on the psychomotor activity of mice. *Nature, Lond.*, **193**, 590–591.

FUXE, K. & LJUNGGREN, L. (1965). Cellular localization of monoamines in the upper brain stem of the pigeon. *J. comp. Neurol.*, **125**, 355–382.

GLOWINSKI, J. & IVERSEN, L. L. (1966). Regional studies of catecholamines in the rat brain—I: The disposition of [^{3}H]-norepinephrine, [^{3}H]-dopamine and [^{3}H]-dopa in various regions of the brain. *J. Neurochem.*, **13**, 655–669.

GLOWINSKI, J., KOPIN, I. J. & AXELROD, J. (1965). Metabolism of [^{3}H]-norepinephrine in the rat brain. *J. Neurochem*, **12**, 25–30.

GROSSMAN, S. P. (1968). Behavioural and electroencephalographic effects of micro-injections of neurohumors into the midbrain reticular formation. *Physiol. Behav.*, **3**, 777–786.

GRUNDEN, L. R. (1969). Action of intracerebroventricular epinephrine on gross behaviour, locomotor activity and hexobarbital sleeping times in rats. *Int. J. Neuropharmac.*, **8**, 573–586.

GRUNDEN, L. R. & KATZUNG, B. G. (1964). Studies on the central action of large doses of epinephrine. *Fedn Proc.*, **23**, 455.

GRUNDEN, L. R. & MARLEY, E. (1970). Effects of sympathomimetic amines injected into the third ventricle in adult chickens. *Neuropharmacology*, **9**, 119–128.

HARRISON, T. S. (1961). Some factors influencing thyrotropin release in the rabbit. *Endocrinology*, **68**, 466–478.

HARVEY, S. C. & NICKERSON, M. (1951). Adrenergic mechanisms in the chicken. *Fedn Proc.*, **10**, 307.

HERTTING, G. (1964). The fate of ^{3}H-*iso*-proterenol in the rat. *Biochem. Pharmac.*, **13**, 1119–1128.

IVY, A. C., GOETZL, F. R., HARRIS, S. C. & BURRILL, D. Y. (1944). The analgesic effect of intracarotid and intravenous injection of epinephrine in dogs and of subcutaneous injection in man. *Q. Bull. N. West. Univ. med. Sch.*, **18**, 298–306.

JUORIO, A. V. & VOGT, M. (1967). Monoamines and their metabolites in the avian brain. *J. Physiol., Lond.*, **189**, 489–518

KANEMATSU, S., KII, M., SONODA, T. & KATO, Y. (1967). Effects of hypothalamic lesions on body temperature in the chicken. *Jap. J. vet. Sci.*, **29**, 95–104.

KEY, B. J. & MARLEY, E. (1962). The effect of the sympathomimetic amines on behaviour and electrocortical activity of the chicken. *Electroenceph. clin. Neurophysiol.*, **14**, 90–105.

KUNTZMAN, R., COSTA, E., CREVELING, C. R., HIRSCH, C. & BRODIE, B. B. (1962). Inhibition of norepinephrine synthesis in mouse brain by blockade of dopamine β-oxidase. *Life Sci., Oxford*, **1**, 85–92.

LEIMDORFER, A. (1950). The action of sympathomimetic amines on the central nervous system and the blood sugar. Mechanism of action. *J. Pharmac. exp. Ther.*, **98**, 62–71.

LEIMDORFER, A. & METZNER, W. R. T. (1949). Analgesia and anesthesia induced by epinephrine. *Am. J. Physiol.*, **157**, 116–121.

LEIMDORFER, A., ARANA, R. & HACK, M. A. (1947). Hyperglycemia induced by the action of adrenaline on the central nervous system. *Am. J. Physiol.*, **150**, 588–595.

MARLEY, A. & PAYNE, J. P. (1964). Halothane anaesthesia in the fowl. In: *Small Animal Anaesthesia*, ed. Graham-Jones, O., pp. 127–134. Oxford: Pergamon Press.

MARLEY, E. & STEPHENSON, J. D. (1968a). Intracerebral micro-infusions of amines in young chickens. *J. Physiol., Lond.*, **196**, 116–117*P*.

MARLEY, E. & STEPHENSON, J. D. (1968b). Intracerebral micro-infusions of drugs under controlled conditions in young chickens. *J. Physiol., Lond.*, **196**, 97–99*P*.

MARLEY, E. & STEPHENSON, J. D. (1969). Effects of some catecholamines infused into the hypothalamus of young chickens. *Br. J. Pharmac.*, **36**, 194*P*.

MASUOKA, D. & HANSSON, E. (1967). Autoradiographic distribution studies of adrenergic blocking agents. II. ^{14}C-Propranolol, a β-receptor type blocker. *Acta Pharmac. Tox.*, **25**, 447–455.

MYERS, R. D. (1964). Emotional and autonomic responses following hypothalamic chemical stimulation. *Can. J. Psychol.*, **18**, 6–14.

OSWALD, E. O. & STRITTMATTER, C. F. (1963). Comparative studies in the characterization of monoamine oxidases. *Proc. Soc. exp. Biol.*, **114**, 668–673.

PALMER, A. C. (1959). Injection of drugs into the cerebral ventricle of sheep. *J. Physiol., Lond.*, **149**, 209–214.

PSCHEIDT, G. R. & HIMWICH, H. E. (1965). Chicken brain amines: normal levels and effect of reserpine and monoamine oxidase inhibitors. In: *Progress in Brain Research*, vol. **16**, *Horizons in Neuro-psychopharmacology*, ed. Himwich, W. A. & Schadé, J. P., pp. 245–249. Amsterdam: Elsevier.

REITTER, H. (1957). Narkoseeffekt durch intrazisternale Adrenalin Injektion. *Anaesthetist*, **6**, 131–135.

RICHARDS, A. N. & COLLISON, L. W. (1928). An apparatus for the continuous recording of the oxygen consumption of small animals. *J. Physiol., Lond.*, **66**, 299–306.

ROSS, S. B. & RENYI, A. L. (1966). Uptake of some tritiated sympathomimetic amines by mouse brain cortex slices *in vitro*. *Acta Pharmac. Tox.*, **24**, 297–309.

ROTHBALLER, A. B. (1959). The effects of catecholamines on the central nervous system. *Pharmac. Rev.*, **11**, 494–547.

SCHMIDT, J. & FÄHSE, C. (1964). Die Wirkung von Hemmstoffen der Monoaminoxydase auf die Körpertemperatur von Ratten. *Acta Biol. med. germ.*, **13**, 607–614.

SCHÜMANN, H. J. & GROBECKER, H. (1965). Über die Wirkung von α-Methyl-Dopa auf den Brenzcatechinamingehalt von Meerschweinchenorganen. *Arch exp. Path. Pharmak.*, **251**, 48–61.

SPOONER, C. E. & WINTERS, W. D. (1965). Evidence for a direct action of monoamines on the chick central nervous system. *Experientia*, **21**, 256–258.

SPOONER, C. R., WINTERS, W. D. & MANDELL, A. J. (1966). DL-norepinephrine-7-H^3 uptake, water content, and thiocyanate space in the brain during maturation. *Fedn Proc.*, **25**, 451.

STEPHENSON, J. D. (1969). Actions of sympathomimetic and allied amines on the avian central nervous system. Ph.D. thesis, University of London.

STOCK, K. & WESTERMANN, E. (1966). Competitive and non-competitive inhibition of lipolysis by α- and β-adrenergic blocking agents, methoxamine derivatives, and prostaglandin E_1. *Life Sci., Oxford*, **5**, 1667–1678.

STRÖM, G. (1960). Central nervous regulation of body temperature. In: *Handbook of Physiology*, Section I, *Neurophysiology*, vol. **2**, pp. 1173–1196, ed. Field, J., Magoun, H. W. & Hall, V. E. Washington: Amer. Phys. Soc.

TRACZYK, W. Z. (1964). Experimental catalepsy produced in dogs by intraventricular administration of adrenaline and noradrenaline. *Int. J. Neuropharmac.*, **3**, 261–266.

UDENFRIEND, S. & ZALTZMAN-NIRENBERG, P. (1962). On the mechanism of norepinephrine release by α-methyl-meta-tyrosine. *J. Pharmac. exp. Ther.*, **138**, 194–199.

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