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ELECTROCORTICAL CHANGES INDUCED BY THE PERFUSION OF NORADRENALINE, ACETYLCHOLINE AND THEIR ANTAGONISTS DIRECTLY INTO THE DORSAL RAPHÉ NUCLEUS OF THE CAT

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1 The electrocortical changes induced by the perfusion of drugs directly into the dorsal raphé nucleus of the cat *encéphale isolé* preparation have been studied.

2 (–)-Noradrenaline (NA), (–)-adrenaline, or (–)-isoprenaline (Isop) produced intermittent or sustained electrocortical desynchronization during the perfusion period.

3 These changes were markedly attenuated or completely abolished by the prior perfusion of (±)-sotalol or (–)-propranolol, but not by equimolecular concentrations of (+)-propranolol.

4 The effects of NA or Isop were also blocked by phentolamine, whereas phenoxybenzamine either potentiated the responses to NA and Isop or mimicked the effects of these catecholamines.

5 The effect of dopamine was similar to that induced by NA, but could not be elicited at all of the perfusion sites where NA was effective. It could be blocked by (±)-sotalol or (–)-propranolol and also by the prior perfusion of fusaric acid.

6 Acetylcholine (ACh) increased, or initiated, electrocortical synchronization. These effects could be antagonized by sensory stimulation, the prior perfusion of atropine, or the simultaneous perfusion of NA at the same site.

7 Lignocaine, induced prolonged electrocortical desynchronization, behavioural alerting and an increased responsiveness to sensory stimulation.

8 The results have been discussed in relation to the possible involvement of inhibitory β-adrenoceptors and facilitatory cholinoceptors (muscarinic) in the functioning of the dorsal raphé nucleus.

Introduction

Of the rostrally projecting midline raphé nuclei, nucleus raphé dorsalis (NRD) possesses a high density of 5-hydroxytryptamine-containing cells (Dahlstrom & Fuxe, 1965) and contributes a significant proportion of its fibres to the forebrain and, in particular, to neocortical structures (Lorens & Guldberg, 1974). It is thus well suited anatomically to fulfil a major role in the modulation of electrocortical activity. Indeed, although lesions confined to this structure do not produce the severity of behavioural deficit incurred on destruction of other raphé nuclei (Srebro & Lorens, 1975), the NRD would appear to play an important part in electrocortical synchronization and sleep mechanisms (Morgane & Stern, 1972; Jouvet, 1973).

In view of these observations it was thought worthwhile to study the influence on the arousal level and patterns of electrocortical activity of substances applied directly to neurones within the DRN through stereotactically implanted perfusion cannulae. A number of putative transmitter substances have been implicated in the functioning of the raphé system (Couch, 1970) and fluorescence and histochemical studies have indicated that the nuclei receive both noradrenergic and cholinergic afferent inputs (Lewis & Shute, 1967; Chu & Bloom, 1974; Saavedra, Grobecker & Zivin, 1976). The present paper therefore deals with the effects of noradrenaline (NA) and acetylcholine (ACh) and their respective antagonists, in an attempt to determine their role within the DRN and the character of the receptors involved.

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Methods

A total of 29 cat *encéphale isolé* preparations were used. The operative procedure, carried out under halothane/O_2 anaesthesia, the type and position of the cortical recording electrodes, as well as the implantation technique have been described previously (Bradley & Elkes, 1957). A concentric, two-tube perfusion cannula (Key, 1975), angled at 45°, was inserted stereotactically using co-ordinates from the cat brain atlas of Snider & Niemer (1961), so that the tip of the outer cannula lay just below the ventricular surface in the dorsal aspect of the nucleus raphé dorsalis. The area of perfusion at the tip of the cannula was 0.53 mm^2. Drugs were dissolved in artificial cerebro-spinal fluid (CSF) and perfused at a rate of 120 µl/min with a 20-channel Watson-Marlow H.R. Flow Inducer. The artificial CSF was perfused continuously throughout the experiment and the drug solutions switched in for 5 min periods.

Systemic blood pressure was monitored by means of a mercury manometer connected to the femoral artery. Wound edges and pressure points were infiltrated with 1% w/v lignocaine hydrochloride solution upon completion of all operative procedures and subsequently at intervals throughout the experiment. The animal was then released from the stereotactic apparatus, the anaesthetic discontinued and a period of 1 h allowed for recovery from the effects of the anaesthetic. Control recordings of electrocortical activity were taken over a further hour before the perfusion of artificial CSF was started.

The electrocorticogram (ECoG) was monitored throughout the experiment and for descriptive purposes reference has been made to four basic patterns representing the alert, relaxed, drowsy and sleeping behavioural states (Figure 1b) according to the schema described by Bradley & Elkes (1957). Rapid eye movement (paradoxical) sleep was not observed in these preparations. A more quantitative assessment of the changes produced in the ECoG was carried out by integrating the electrocortical waveform recorded from the association cortex of the left or right middle supra-sylvian gyrus. The output of the integrator was in the form of pulses representing fixed increments of electrical energy. These pulses were recorded on the ECoG coincident with the appopriate electrical activity (Figure 1b-int). The data were further analysed by counting the number of pulses for each consecutive 20 s period and expressing these cumulative integrals graphically to reflect the changing levels of desynchronization of the ECoG (Figure 1a). Upon completion of the experiment the brain was removed and fixed in 10% formol saline solution for later histological investigation.

All experiments were carried out in the open laboratory where levels of ambient, and especially auditory, stimulation varied. Since this factor appeared to be important in the intrepretation of the results, an assessment of this variation was made at the time of the experiment. Under 'quiet' conditions sound levels rarely exceeded 20 dB (measured relative to 0.0002 dynes cm^2 at the position of the animal). Moderate levels, usually of continuous noise varied up to 50 dB, but occasionally higher levels of an intermittent nature were registered above this level.

The drugs tested were atropine sulphate (BDH), acetylcholine chloride (BDH), phentolamine mesylate (Ciba), fusaric acid (Sigma), and the hydrochlorides of (–)-adrenaline (Sigma), dopamine (Koch-Light), (–)-isoprenaline (Sigma), (–)-noradrenaline (Sigma), phenoxybenzamine (SKF), (–)- and (+)-propranolol (ICI), (±)-sotalol (Mead-Johnson) and lignocaine (Antigen).

Results

Control recordings of electrocortical activity showed that no observable changes in the patterns occurred when the temperature of the perfusate was maintained at 38° ± 1.5°C and the pH between 5.7 to 7.2. The tube from the output cannula was open-ended and the flow of perfusate maintained by the positive pressure of the pump. Because the tip of the inner input tube was 0.5 mm shorter than the outer tube, the resistance to the flow of perfusate from input to output systems was never greater than 1 mmHg. Thus, brain deformation at the tip of the cannula was insigificant and dye studies carried out at the end of the experiments indicated that although in each case the cannulae were in the midline and only 0.5–0.75 mm below the ventricular surface, no leakage of perfusate occurred back along the outside of the cannula into the aqueduct or fourth ventricle. In the experiments which are described all the above criteria were fulfilled and histological examination showed that tissue damage was limited to that incurred on insertion of the cannula.

Noradrenaline

Noradrenaline (NA) in the concentration range 10^{-6}–10^{-3}M was perfused in 29 cats. In the drowsy or sleeping animal, where the ECoG showed varying degrees of synchronization, perfusion of NA above 10^{-1}M always induced some measure of desynchronization. These effects are illustrated in Figure 1, where an increase in electrocortical desynchronization is reflected by a decrease in the integral counts. The effects of NA were dose-related. Concentrations of 10^{-5}M induced slight increases in the frequency of electrocortical activity, whereas concentrations of 5×10^{-5}M or above produced short, 1–10 s, periods of desynchronization (phasic arousal responses) after 40–90 s of perfusion. This pattern then either persisted for the duration of the perfusion or was replaced by prolonged desynchronization associated

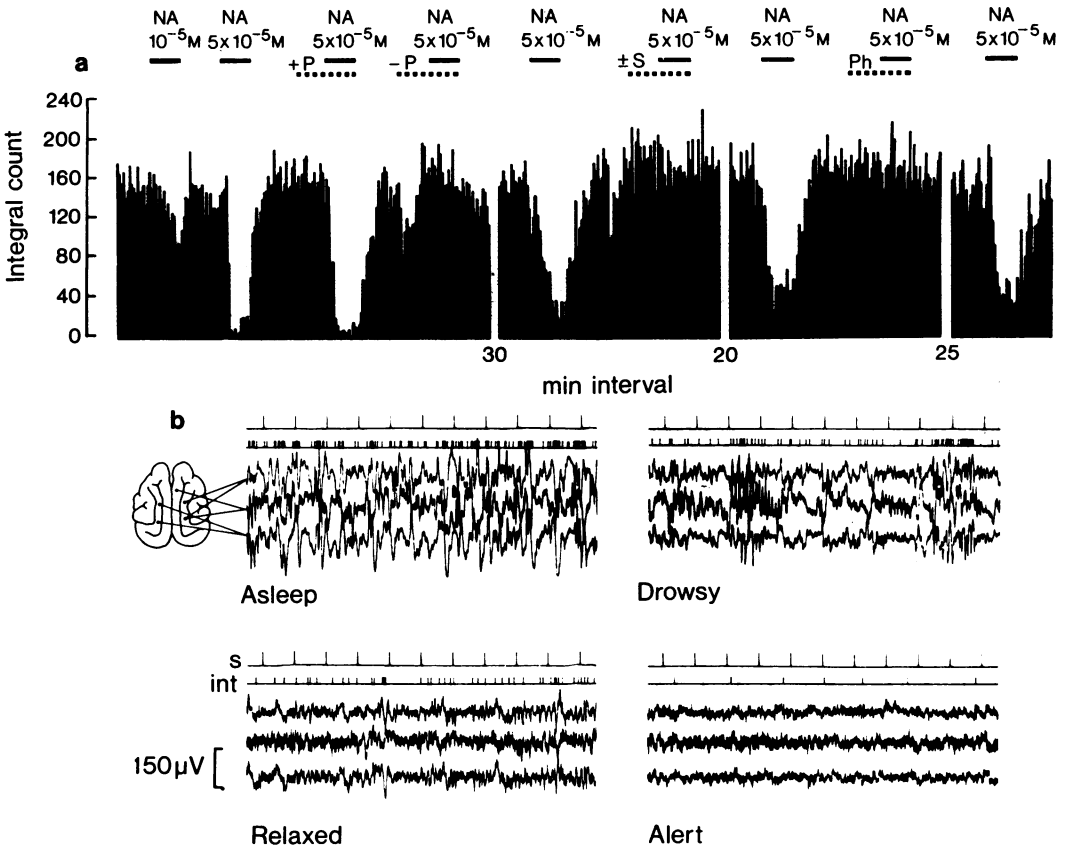

Figure 1 The effect of noradrenaline (NA) on the pattern of electrocortical activity when perfused into the dorsal raphé nucleus of the cat. (a) Histogram depicting the integral counts over successive 20 s periods. Solid bars show 5 min perfusion periods of NA. Perfusion of NA antagonists indicated by the interrupted bars. Desynchronization of the electrocorticogram, recorded from the right middle suprasylvian gyrus is reflected by a decrease in the integral counts. Note the dose-related changes induced by NA with the subsequent blockade of the response by (–)-propranolol (–P, 5×10^{-5} M), (±)-sotalol (S, 5×10^{-5} M) and phentolamine (Ph, 5×10^{-4} M) but not by (+)-propranolol (+P, 5×10^{-5} M). In this experiment (–)-propranolol induced a transient desynchronization of the ECoG at the beginning of the perfusion period. (b) Examples of the four basic patterns of electrocortical activity referred to in the text with the coresponding integral pulses (int). Integration was carried out on the electrical record taken from the right middle suprasylvian gyrus (top trace in each record).

with behavioural arousal (tonic arousal response). However, the appearance of tonic responses could be blocked or at least delayed, by a reduction in the level of ambient sensory stimulation. Recovery from the effects of NA were extremely variable. The majority of animals returned to the sleeping state within 1–10 min, while others, once tonic arousal had occurred, remained alert or in the relaxed behavioural state, for up to 40 minutes.

Perfusion of adrenaline (3 expts) or isoprenaline (Figure 2—Isop, 10 experiments) produced effects comparable to those of NA, but the minimal effective concentrations were slightly higher (5×10^{-5} M). Tachyphylaxis occurred to all three drugs, especially with high concentrations (10^{-3} M) and when the drugs were applied at intervals shorter than 5 minutes. No consistent blood pressure changes were noted on perfusion of adrenaline, NA or Isop.

Adrenoceptor antagonists

The β-adrenoceptor antagonists (–)-propranolol and (±)-sotalol blocked or significantly attenuated the phasic and tonic electrocortical responses induced by both NA and Isop. Both drugs showed similar antagonistic activity and were effective in concentrations as low as 10^{-5} M, providing they were applied during, and at least 5 min before the perfusion

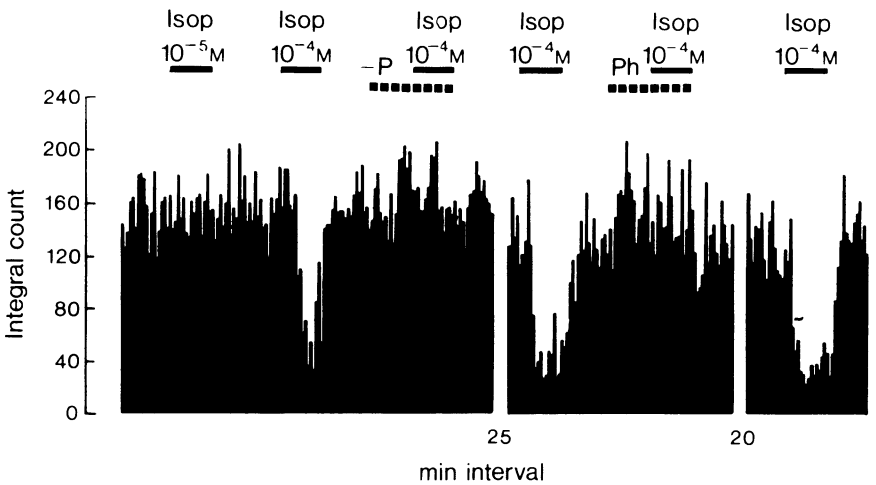

Figure 2 Histogram showing the effect of isoprenaline (Isop) on the pattern of electrical activity when perfused into the dorsal raphé nucleus of the cat. Integral counts taken over successive 20 s periods using electrocortical activity of the right middle suprasylvian gyrus taken from the same experiment as in Figure 1. Solid bars indicate 5 min perfusion periods of isoprenaline. Perfusion of antagonists shown by interrupted bars (15 minutes). Note response of Isop blocked by (−)-propranolol (−P, 5×10^{-4} M) and phentolamine (Ph, 5×10^{-4}M).

of NA or Isop (Figures 1 and 2). Basic differences in the responses of the two antagonists were apparent, since (−)-propranolol appeared to possess some noradrenergic agonist activity. In the sleeping animal concentrations of at least 5×10^{-5} M occasionally produced an overall increase in the frequency of electrocortical activity or induced short periods of desynchronization. These effects were transient and usually only apparent in the first 1–3 min of the perfusion as shown by a decrease in the cumulative integral count (Figure 1a). It is unlikely that these changes are related to local anaesthetic activity, since an equimolar concentration of the (+)-isomer of propranolol had no effect (Figure 1a). Indeed, by comparison with the responses induced by the local anaesthetic lignocaine (Figure 6), the concentrations of (−)- or (+)-propranolol that appeared to have local anaesthetic activity and produce long-lasting electrocortical desynchronization, had to be at least 10^{-3} M. This effect then persisted for 10–30 min after the end of the perfusion period.

Phenoxybenzamine (10^{-6}–10^{-4} M) failed to antagonize the response induced by NA. At a concentration of 10^{-5} M this drug induced short 1–5 s bursts of electrocortical desynchronization and even at 5×10^{-6} M potentiated rather than blocked the effect of subsequent applications of NA or Isop. With concentrations in excess of 10^{-5} M phenoxybenzamine produced tonic electrocortical and behavioural arousal responses. In contrast, phentolamine (10^{-5}–10^{-3} M) had no effect on the ECoG but when used in concentrations of 10^{-4} M or above, consistently attenuated or completely blocked the responses induced by NA and Isop (Figures 1 and 2). However, when the antagonistic action of (−)-propranolol was compared with that of phentolamine in the same preparation (Figure 1), it was always found to be at least 10 times more potent as a blocking agent.

Dopamine

Dopamine was perfused in 12 experiments but produced changes in the ECoG in only 8. The electrocortical effects were similar to those of NA (Figure 3), but noticeably, much higher perfusate concentrations (10^{-4}–5×10^{-3}M) were required and consecutive applications, even at widely spaced time intervals, did not yield the same degree of electrocortical desynchronization. Although these observations do not exclude the participation of post-synaptic dopamine receptors, the longer latency and inconsistent nature of the response appeared to favour a non-specific or indirect effect, possibly mediated through NA. This latter hypothesis was investigated using the β-adrenoceptor antagonists, (−)-propranolol and (±)-sotalol, both of which proved effective in 10^{-5}M concentrations in abolishing the electrocortical changes induced by application of dopamine. In addition, prior perfusion for 15 min of the dopamine β-hydroxylase inhibitor, fusaric acid, at a concentration of 10^{-5} M, also blocked the effect of dopamine but not that of NA (Figure 3).

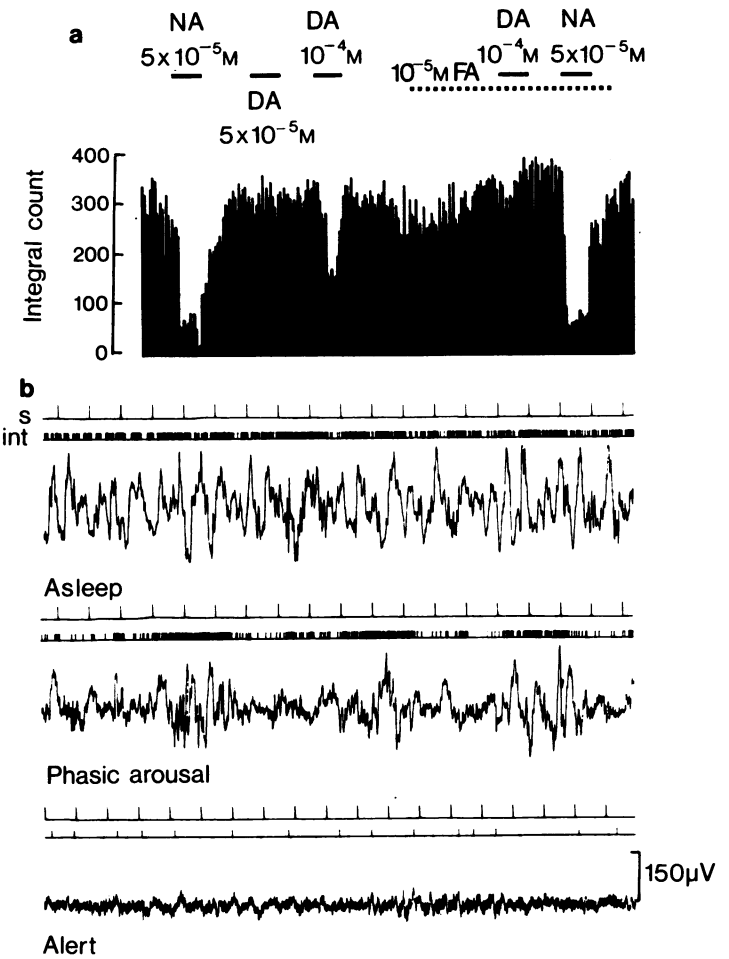

Figure 3 Comparison between the electrocortical changes induced by noradrenaline (NA) and dopamine (DA) when perfused into the same site within the dorsal raphé nucleus of the cat. (a) Histogram showing integral counts over successive 20 s periods. Electrical activity recorded from the right middle suprasylvian gyrus. Solid bars show 5 min perfusion periods of either NA or dopamine. Perfusion of the dopamine β-hydroxylase inhibitor, fusaric acid (FA 10^{-5} M) indicated by the interrupted bars (total time 33 minutes). Note the blockade of the desynchronization induced by dopamine but not that produced by NA. (b) Sample electrocortical records taken from the right middle suprasylvian gyrus with concomitant integral pulses (int). Record of electrocortical activity when the animal was asleep taken immediately before the initial perfusion of dopamine at 10^{-4} M. Phasic arousal responses were obtained during the perfusion of dopamine (10^{-4} M) and the fully desynchronized record (alert) shows the effect of initial perfusion of NA (5×10^{-5} M).

Acetylcholine

The effect of acetylcholine (ACh) was studied in 15 preparations and found to be opposite to that of NA. To produce a change in electrocortical activity it was found necessary to perfuse ACh in high concentrations (10^{-3}–10^{-1} M). If physostigmine (10^{-5} M) was added to the perfusage (3 experiments), the effective concentrations of ACh were considerably lower (10^{-5}–10^{-4} M). However, in view of the observations of Bradley, Dhawan & Wolstencroft (1966) that some apparently non-cholinoceptive neurones within the CNS respond to iontophoretically applied physostigmine, it was decided to use ACh alone and thus eliminate any misinterpretations which might rise due to the addition of an extra drug. Although ACh was capable of initiating or increasing electrocortical synchronization in the alert animal, the degree of electrical change was determined by the level of ambient sensory stimulation at the time of the perfusion. With high environmental noise levels, especially if the auditory stimuli were of an

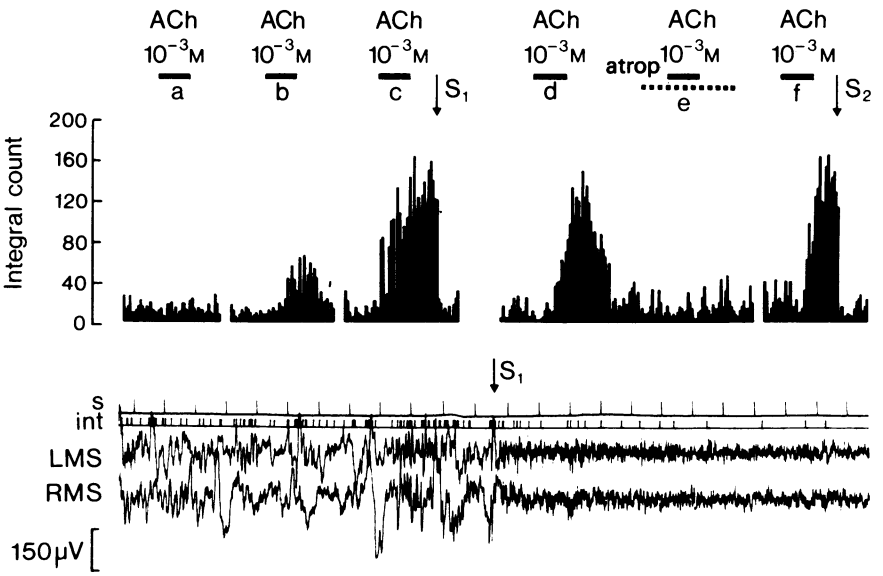

Figure 4 The effect of acetylcholine (ACh) on the patterns of electrocortical activity when perfused into the dorsal raphé nucleus of the cat. Histogram represents integral counts over successive 20 s periods of electrical activity recorded from the left middle suprasylvian gyrus. Solid bars indicate 5 min perfusion periods of ACh. Interrupted bars represent 15 min perfusion period of atropine (Atrop, 10^{-4} M). (a) Lack of effect of ACh when perfused at a time of high ambient noise levels in the immediate environment. (b and c) Electrocortical synchronization induced by the perfusion of ACh with the animal in an environment providing only low to moderate (15–40 dB) levels of ambient noise. (c) Blockade of the electrocortical synchronizing effect of ACh (d) by atropine (Atrop, 10^{-4} M) with subsequent recovery (f). Note that electrocortical desynchronization can easily be evoked by an auditory stimulus (S_1 and S_2) after ACh had produced synchronization.

intermittent nature, ACh appeared to be without effect (Figure 4a). On the other hand, low to moderate levels of noise (15–40 dB), which were usually sufficient to maintain wakefulness before drug application, proved insufficient after the perfusion of ACh. The changes in the ECoG varied from the introduction of rhythmic (8–12 Hz) activity characteristic of the relaxed behavioural state (Figure 4b), to the initiation of high amplitude, slow waves (150–300 μV, 2–4 Hz) associated with behavioural sleep (Figure 4c).

The effects of ACh could be blocked either by the simultaneous perfusion of NA (10^{-4} M—4 experiments), or the prior perfusion of atropine (5 experiments) at concentrations of 5×10^{-5} M or 10^{-4}M (Figure 4e). The dose-response characteristics for atropine (Figure 5) revealed that at these levels the drug did not exert any significant local anaesthetic activity. In the sleeping animal a concentration of 5×10^{-5} M had no effect on the ECoG (Figure 5). At 10^{-4}M there was a decrease in the cumulative integral count, induced as a result of the introduction of phasic, 5–10 s bursts of desynchronization (Figure 5). This change was not accompanied by any overt behavioural response and terminated quickly (1–3 min) after the end of the perfusion. In contrast, application of concentrations in excess of 5×10^{-4} M (Figure 5) invariably induced prolonged, tonic electrocortical desynchronization and behavioural arousal, occasionally of rather sudden onset (Figure 5–10^{-3} M).

Lignocaine

Since a number of the drugs used in the present study have been reported to possess local anaesthetic activity, especially in high concentrations, it was thought worthwhile to study the effect of the local anaesthetic, lignocaine. Such observations would also provide an indication of the electrocortical and behavioural changes accompanying reversible blockade of the NRD in the *encéphale isolé* preparation. Lignocaine produced tonic electrocortical desynchronization and behavioural arousal within the 5 min perfusion period when applied in concentrations of 10^{-5} M, or above. The effects were usually of sudden onset, long-lasting and with the higher concentrations, characterized by a very short latency (Figure 6–10^{-4} M). It was also noticeable that the animals showed increased responsiveness to sensory stimuli and would respond with head, eye and ear movements to the slightest sound, movement or tactile stimulus. Reduction in the level of ambient

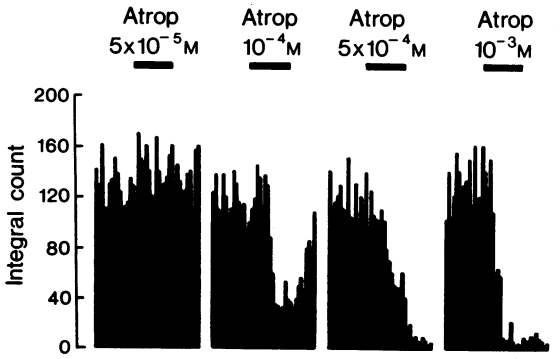

Figure 5 Dose-related changes in the degree of electrocortical desynchronization induced by atropine (Atrop) when perfused into the dorsal raphé nucleus of the cat. Histogram shows integral counts representing successive 20 s periods of electrical activity recorded from the left middle suprasylvian gyrus. Same experiment as in Figure 4. Solid bars represent 5 min perfusion periods of atropine with 20 min recovery periods between each dose. Note response to 5×10^{-4} M atropine shows a two stage effect, while at 10^{-3} M the response has a sudden onset and relatively short latency.

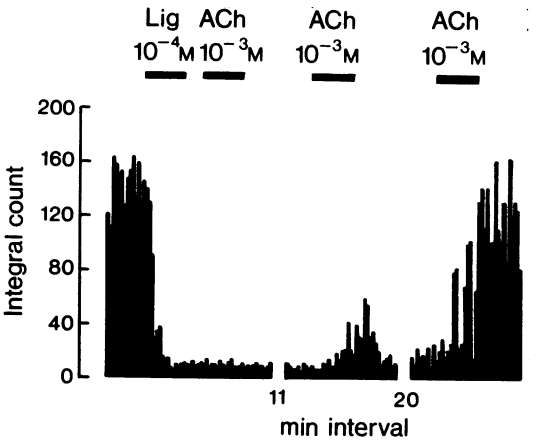

Figure 6 Histogram showing the desynchronizing effect of lignocaine (Lig) when perfused for 5 min into the dorsal raphé nucleus of the cat. Same experiment and perfusion site as in Figures 4 and 5. Note the sudden onset of the response and the blockade of the electrocortical synchronizing effect of acetylcholine (ACh).

sensory stimulation, although usually failing to modify the response once present, invariably delayed its onset.

Lignocaine blocked the synchronizing effects of ACh and recovery, as can be seen from Figure 6, was delayed. Partial ACh responses were apparent after 11 min but full recovery, following a 10^{-4} M concentration of lignocaine, was only seen after a further 20 min (Figure 6).

Discussion

The present study shows that it is possible to influence the arousal level and the pattern of electrocortical activity by application of drugs directly to the dorsal raphé nucleus. NA invariably produced some degree of electrocortical desynchrony when perfused in concentrations of 10^{-5}–10^{-3} M. When considered in terms of the total amount of NA applied during the 5 min perfusion period, these concentrations represent relatively substantial doses. However, similar perfusion studied within the mesencephalic reticular formation have shown that only a fraction of the applied NA, in the order of 0.39–119.2 ng respectively, will actually penetrate into the brain tissue (Key, 1975). On this evidence it would appear that the diffusibility of NA is either very low or that the use of a continuous perfusion process reduces the amount of NA which physically comes into contact with the brain. In either case the amount of NA within the vicinity of individual neurones, especially those located towards the limit of the diffusional area, must be relatively small.

Biochemical, stimulation and lesion studies have indicated that the raphé nuclei play a part in sleep processes and that it is activation of this system which initiates or increases the synchronization of electrocortical activity (Kowstowski, Giacalonne, Garattini & Valzelli, 1969; Morgane & Stern, 1972; Jouvet, 1973). The desynchronization induced by the perfusion of NA would suggest, by analogy, that the predominant effect of this drug within the DRN is one of inhibition. It is also likely on the basis of the results obtained with Isop and adrenaline, that this inhibitory effect is related to β-adrenoceptor activation. The failure of phenoxybenzamine to block the desynchronization induced by NA and Isop would appear to substantiate this view. Even in relatively low concentrations phenoxybenzamine produced changes in the ECoG qualitatively similar to those of NA. Moreover, pretreatment with low concentrations of phenoxybenzamine potentiated rather than blocked the effects of NA or Isop. It has been reported that phenoxybenzamine can facilitate the release (Potter, Chubb, Put & Schaepdryver, 1971), or inhibit the uptake of catecholamines into adrenergic nerve terminals (Iversen, Salt & Wilson, 1972). Both effects, since they are related to pre-synaptic events, would be expected to facilitate the effect of exogenously applied NA and Isop, especially at β-adrenoceptor sites where the antagonistic effects of phenoxybenzamine should be negligible.

Surprisingly, both the β-antagonist, (–)-propranolol and the α-antagonist, phentolamine, attenuated or

abolished the responses induced by NA and Isop. Local anaesthetic effects may be excluded at the dose levels employed since (+)-propranolol, which has greatly reduced β-adrenoceptor blocking activity but is equipotent with the (−)-isomer in terms of local anaesthetic activity (Barrett & Cullum, 1968), did not affect the ability of NA or Isop to produce desynchrony. Moreover, the β-antagonist (±)-sotalol, which is purported to have little or no local anaesthetic activity (Lish, Weikel & Dungan, 1965), was just as effective as (−)-propranolol as a blocking agent. The similarity in effect between phentolamine and the β-antagonists would imply either that it is difficult to categorize the catecholamine receptors in the raphé on the basis of criteria used for the peripheral nervous system, or more likely that the effect of phentolamine, although exerted on β-receptors, was non-specific. Indeed, by comparison with propranolol and sotalol, it was noticeable that higher concentrations of phentolamine were always required. Recently it has been pointed out that catecholamine receptor designation based solely on phentolamine inhibition is to be approached with caution. This drug, especially in high concentration is capable of displacing the specific β-noradrenergic antagonist (−)-alprenolol, from post-synaptic binding sites (Alexander, Davis & Lefkowitz, 1975). In addition, phentolamine is known to produce non-specific inhibitory effects on certain β-adrenergic physiological responses (Moran & Perkins, 1961) and on β-adrenoceptor-coupled adenylate cyclase activity (Vatner & Lefkowitz, 1974).

Although there are no reports of dopamine-containing terminals within the DRN, or any direct evidence of afferent fibre projections to the DRN from dopamine-containing neurones, it was still possible by the perfusion of dopamine to produce electrocortical desynchronization. This effect was not observed in all experiments and only after doses substantially higher than those of NA. It could be blocked by the application of (−)-propranolol or (±)-sotalol, and the prior perfusion of the dopamine β-hydroxylase inhibitor, fusaric acid (Nagatsu, Tekeshi & Hiroshi, 1973), abolished the response in all cases. On this evidence it may be concluded that the dopamine effect was mediated through NA post-synaptic receptor activation but involved initially the pre-synaptic enzymatic conversion of dopamine to NA.

Provided the animal was kept in an environment yielding only a low to moderate level of ambient sensory stimulation, the perfusion of ACh initiated, or increased the degree of electrocortical synchronization. In this respect the response induced by ACh is comparable to that evoked by low frequency electrical stimulation of the raphé system (Kowstowski *et al.*, 1969). However, the effect could be blocked or attenuated by the simultaneous perfusion of NA or by the prior perfusion of atropine. These results would suggest that the cells of the DRN receive a noradrenergic inhibitory input, physiologically antagonistic to an afferent excitatory cholinergic system. On the basis of the blocking action of atropine the post-synaptic cholinoceptors may be classified as of the muscarinic type. Cholinergic involvement in the functioning of the raphé nuclei has been indicated previously by a number of histological and histochemical studies (Lewis & Shute, 1967; Palkovits & Jacobwitz, 1974; Cheney, LeFevre & Racagni, 1975). Recently it has also been shown that [^{3}H]-quinillidinyl-benzilate (QNB), a potent cholinoceptor antagonist, bind specificially to muscarinic post-synaptic receptors (Yamamura, Kuhar & Snyder, 1974). Within the midbrain, binding has been observed in the raphé area and in particular within the dorsal midbrain raphé (Kuhar & Yamamura, 1975).

The behavioural and electrical changes produced by the perfusion of NA and ACh into the DRN were clearly related to environmental conditions. On the evidence presented, the effect of NA was predominantly one of inhibition. Even so, in the sleeping animal blockade of dorsal raphé activity was usually insufficient to bring about tonic behavioural and electrocortical arousal unless some degree of ambient sensory stimulation was present. Since this level of stimulation was ineffective before drug application, it would appear that the alerting was induced by the same sensory input to which the animal, as a result of raphé inhibition, had become more susceptible. In this context it is worth noting that the raphé nuclei have neural connections, or exert inhibitory influences, not only on the hypothalamus, neocortex, limbic system and the reticular formation, but also on structures concerned with the specific sensory systems (Fuxe, 1965; Nakamura, 1975; Bobillier, Sequin, Petitjean, Salvert, Touret & Jouvet, 1976). Moveover, the raphé nuclei have been implicated in behavioural functions related to changes in sensory responsiveness or the integration of sensory information (Sheard & Aghajanian, 1969; Geyer, Puerto, Menkes, Segal & Mandell, 1976). The raphé nuclei therefore, instead of forming part of an active sleep mechanism, may provide a system which controls the flow and integration of sensory information. Sleep, induced as a result of raphé activation, could thus be an indirect effect initiated by the modulation of sensory inflow and the establishment of conditions conducive to the production of sleep.

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References

ALEXANDER, R.W., DAVIES, J.N. & LEFKOWITZ, R.J. (1975). Direct identification and characterization of β-adrenergic receptors in rat brain. *Nature, Lond.*, **258**, 437–440.

BARRETT, A.M. & CULLUM, V.A. (1968). The biological properties of optical isomers of propranolol and their effects on cardiac arrhythmias. *Br. J. Pharmac.*, **34**, 43–55.

BOBILLIER, P., SEQUIN, S., PETITJEAN, F., SALVERT, D., TOURET, M. & JOUVET, M. (1976). The raphé nuclei of the cat brain stem: a topographical atlas of their efferent projections as revealed by autoradiography. *Brain Res.*, **113**, 449–486.

BRADLEY, P.B. & ELKES, J. (1957). The effect of drugs on the electrical activity of the brain. *Brain*, **80**, 77–117.

BRADLEY, P.B., DHAWAN, B.N. & WOLSTENCROFT, J.H. (1966). Pharmacological properties of cholinoceptive neurones in the medulla and pons of the cat. *J. Physiol., Lond.*, **183**, 658–674.

CHENEY, D.L., LEFEVRE, H.F. & RACAGNI, G. (1975). Choline acetyltransferase activity and mass fragmentographic measurement of acetylcholine in specific nuclei and tracts of rat brain. *Neuropharmacology*, **14**, 801–809.

CHU, N-S. & BLOOM, F.E. (1974). The catecholamine-containing neurones in the rat dorso-lateral pontine tegmentum: distribution of the cell bodies and some axonal projections. *Brain Res.*, **66**, 1–21.

COUCH, J.R. (1970). Responses of neurons in the raphé nuclei to serotonin, norepinephrine and acetylcholine and their correlation with an excitatory synaptic input. *Brain Res.*, **19**, 137–150.

DAHLSTROM, A. & FUXE, K. (1965). Evidence for the existence of monoamine-containing neurons in the central nervous system. 1. Demonstration of monoamines in the cell bodies of brain stem neurones. *Acta physiol. scand.*, **62**, Suppl. 232, 1–55.

FUXE, K. (1965). Evidence for the existence of monoamine-containing neurons in the central nervous system IV. Distribution of monoamine nerve terminals in the central nervous system. *Acta physiol. scand.*, **64**, Suppl. 247, 41–85.

GEYER, M.A., PUERTO, A., MENKES, D.B., SEGAL, S.S. & MANDELL, A.J. (1976). Behavioural studies following lesions of the mesolimbic and mesostriatal serotonergic pathways. *Brain Res.*, **106**, 257–270.

IVERSEN, L.L., SALT, P.J. & WILSON, H.A. (1972). Inhibition of catecholamine uptake in the isolated rat heart by haloalkylamines related to phenoxybenzamine. *Br. J. Pharmac.*, **46**, 647–657.

JOUVET, M. (1973). Serotonin and sleep in the cat. In *Serotonin and Behaviour*, ed. Barchas, I. & Usdin, E., pp. 385–400. New York: Academic Press.

KEY, B.J. (1975). Electrocortical changes induced by the perfusion of catecholamines into the brainstem reticular formation. *Neuropharmacology*, **14**, 41–51.

KOWSTOWSKI, W., GIACALONNE, E., GARATTINI, S. & VALZELLI, L. (1969). Electrical stimulation of midbrain raphé: biochemical, behavioural and bioelectric effects. *Eur. J. Pharmac.*, **7**, 170–175.

KUHAR, M.J. & YAMAMURA, H.I. (1975). Light autoradiographic localization of cholinergic muscarinic receptors in rat brain by specific binding of a potent antagonist. *Nature, Lond.*, **253**, 560–561.

LEWIS, P.R. & SHUTE, C.C.D. (1967). The cholinergic limbic system: Projections to hippocampal formation, medial cortex, nuclei of the ascending cholinergic reticular system, and the subfornical organ and supra-optic crest. *Brain*, **110**, 521–540.

LISH, P.M., WEIKEL, J.H. & DUNGAN, K.W. (1965). Pharmacological and toxological properties of two new β-adrenergic receptor antagonists. *J. Pharmac exp. Ther.*, **149**, 161–173.

LORENS, S.A. & GULDBERG, H.C. (1974). Regional 5-hydroxytryptamine following selective midbrain raphé lesions in the rat. *Brain Res.*, **78**, 45–56.

MORAN, N.C. & PERKINS, M.J. (1961). An evaluation of adrenergic blockade of the mammalian heart. *J. Pharmac. exp. Ther.*, **133**, 192–201.

MORGANE, P.J. & STERN, W.C. (1972). Relationship of sleep to neurochemical circuits, biochemistry and behaviour. *Ann. N.Y. Acad. Sci.*, **193**, 95–111.

NAGATSU, T., TEKESHI, K. & HIROSHI, K. (1973). New inhibitors of microbial origin for dopamine-β-hydroxylase. In *Frontiers of Catecholamine Research, ed. Usdin, E. & Synder, S., pp. 87–90. New York: Pergamon Press.*

NAKAMURA, S. (1975). Two inhibitory effects upon brain stem reticular neurons by low frequency stimulation of raphé nucleus in the rat. *Brain Res.*, **93**, 140–144.

PALKOVITS, M. & JACOBWITZ, D.M. (1974). Topographic atlas of catecholamine and acetylcholinesterase — containing neurons in the rat brain. II. Hindbrain (mesencephalon, rhombencephalon). *J. comp. Neurol.*, **157**, 29–42.

POTTER, W.P. DE., CHUBB, I.W., PUT, A. & SCHAEPDRYVER, A.F. DE (1971). Facilitation of the release of noradrenaline and dopamine-β-hydroxylase at low stimulation frequencies by α-blocking agents. *Archs int. Pharmacodyn. Ther.*, **193**, 191–197.

SAAVEDRA, J.M., GROBECKER, H. & ZIVIN, J. (1976). Catecholamines in the raphé nuclei of the rat. *Brain Res.*, **114**, 339–345.

SHEARD, M.H. & AGHAJANIAN, G.K. (1969). Neural release of serotonin: interaction with drugs. In *The Present Status of Psychotropic Drugs*, ed. Cerletti, A. & Bové, F.J., pp. 323–324. Amsterdam: Excerpta Medica Foundation.

SNIDER, R.S. & NIEMER, W.T. (1961). *A Stereotaxic Atlas of the Cat Brain.* Chicago: University of Chicago Press.

SREBRO, B. & LORENS, S.A. (1975). Behavioural effects of selective midbrain raphé lesions in the rat. *Brain Res.*, **89**, 303–325.

VATNER, D.E. & LEFKOWITZ, R.J. (1974). ^{3}H-Propranolol binding sites in myocardial membranes—nonidentity with *beta*-adrenergic receptors. *Mol. Pharmac.*, **10**, 450–456.

YAMAMURA, H.I., KUHAR, M.J. & SNYDER, S.H. (1974). *In vivo* identification of muscarinic cholinergic receptor binding in rat brain. *Brain Res.*, **80**, 170–176.

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