

INHIBITION OF VESTIBULOSPINAL REFLEXES FOLLOWING CHOLINERGIC ACTIVATION OF THE DORSAL PONTINE RETICULAR FORMATION

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INTRODUCTION

After the pioneer work by Hernandez Peon *et al.* (23, 81), who precipitated desynchronized sleep (D) episodes by local application of acetylcholine (ACh) crystals to various brain regions, several studies have implicated cholinergic mechanisms in the generation of D (see 26, 32 for reviews). As a result of these experiments it appeared that infusion of cholinergic agonists into the pontine reticular formation (pRF) of intact cats was able to induce D-like behavior and some or all of the electrophysiological correlates of D, including postural atonia and bursts of rapid eye movements (REM). In particular, D could be elicited by small volume injections in pRF sites of the cholinergic agonists carbachol (1, 75, 82), betanecol (5, 25), or ACh itself (4), and of the acetylcholinesterase (AChE) inhibitor neostigmine (2, 3, 6); the effects were both site-specific and dose-dependent and could be blocked by local injection of the muscarinic receptor antagonist atropine sulphate.

Cholinergic mechanisms of D were also examined by using other preparations and experimental approaches. In particular, some authors (40, 45, 46, 50) have shown that, in precollicular decerebrate cats, i.v. administration of the anti-AChE eserine sulphate produced episodes of muscle atonia accompanied by stereotyped bursts of REM and monophasic waves in the pons (cf. also 32). These eserine-induced D-like episodes were blocked by systemic injection of atropine sulphate (40, 46). The AChE-inhibitor neostigmine methylsulphate and the muscarinic receptor blocker atropine methylnitrate, which do not effectively cross the blood-brain barrier, were not effective. On the basis of these and other findings it was again proposed that brain stem cholinceptive systems play a role in the control of tonic (muscle atonia) as well as phasic (REM) manifestations of D, and that both these events do not require the presence of supramesencephalic structures (64, 65).

It seems reasonable to assume that the episodes of postural atonia occurring in intact animals during D are in fact closely related to those induced by i.v. administration of an anti-AChE in decerebrate cats. For instance, in both cases postural atonia involves extensor as well as flexor muscles of the limbs (16,72) and is associated with a tonic depression of monosynaptic reflexes involving extensor and flexor motoneurons (61-63, 72; see 64, 65 for ref.). Likewise, these effects could be attributed in both instances to descending volleys arising from supraspinal

structures like the pRF, relaying in the medullary reticular formation (mRF) and finally causing postsynaptic inhibition of α -motoneurons (see 65-67 for ref.). This hypothesis is supported by the results of unit recording experiments showing that neurons located in the inhibitory area of the mRF are depolarized or increase their discharge rate during D (13, 15, 33) as well as during the episodes of postural atonia induced by i.v. injection of an anti-AChE (76). Moreover, activation of inhibitory reticulospinal neurons is likely to be responsible for the postsynaptic inhibition which affects α -motoneurons during D (14, 18-20, 52-54) as well as following systemic injections of an anti-AChE (72).

The aim of the present study was to ascertain whether the effects of systemic injections of an anti-AChE, characterized by the suppression of postural activity as well as by the occurrence of rhythmic bursts of REM, could also be elicited, in precollicular decerebrate cats, by microinjection of the cholinergic agonist carbachol into pontine structures and, if so, whether the critical region involved in these phenomena corresponded to the dorsal pontine tegmentum, which according to Sakai (66) includes the locus coeruleus α (LC α), the peri-LC α and the surrounding pRF. Moreover, since a suppression of the vestibulospinal reflexes occurred in decerebrate cats during the episodes of postural atonia induced by systemic injection of an anti-AChE (44), experiments were performed to find out whether similar results were also obtained after local injection of carbachol into pontine tegmental structures.

METHODS

Experiments were performed in 9 precollicular decerebrate cats operated under ether anesthesia. The head of the animal was fixed in a stereotaxic frame and pitched 15° nose-down, while both fore- and hindlimbs were extended and clamped.

Screw electrodes were inserted in the floor of the frontal sinus on each side for recording the electrooculogram (EOG), while appropriate electrodes were used to record the electrocardiogram (EKG). Moreover, pairs of EMG electrodes made of 200 μ m steel wires were inserted in the medial head of the triceps brachii of both sides.

The methods for recording and analysing the multiunit EMG responses of the medial head of the triceps brachii of both sides to roll tilt of the animal at 0.15 Hz, $\pm 10^\circ$ were described in detail in a previous study (8). Groups of 5-6 superimposed sweeps, each containing the responses to two successive cycles (128 bins, with a bin width of 0.1 sec) were averaged and compared at regular intervals of 3 to 6 min for several hours before and after the injection of small doses of the cholinergic agonist carbachol into brainstem structures of one or both sides.

By means of a computer system (PET, 2001-8C), both the rotational stimulus (angular input) and the output (averaged firing rate of the multiunit activity) were evaluated, and the gain, sensitivity and phase angle of the first harmonic of the output with respect to the input were derived by cross-spectral analysis. The gain was expressed in impulses per second per degree of displacement (imp./sec/deg), the sensitivity was expressed as the percentage change of the mean discharge rate per degree of displacement (%/deg), while the base frequency (BF), expressed in imp./sec, corresponded to the DC value obtained from harmonic analysis of the response and was usually comparable to the mean frequency of the multiunit discharge recorded at rest. The phase angle of the first harmonic

response was expressed in arc degrees with respect to the side-down position of the whole animal (downward displacement of the table). A coherence coefficient varying between 0 and 1.0 was also computed to provide a measure of the degree of correlation between the input and the output; a value of 1.0 represents a linear, time-invariant, noise-free system. We considered to be positive only those responses to successive cycles of stimulation that had a coherence coefficient higher than 0.7 and a sensitivity equal to or greater than 0.3%/deg.

Unilateral injections of the cholinergic agonist carbachol into the brainstem were performed through a stainless steel cannula with a tip diameter of 0.5 mm. In particular, in some instances the cannula was lowered into the dorsal aspect of the pontine tegmentum of one side at the stereotaxic coordinates of P3, L2.5-2.8, H-2.0 to -3.0. In these cases 0.05-0.25 μ l of carbachol at the concentration of 0.01-0.2 μ g/ μ l of sterile saline, marked with 5% pontamine, were injected. In other instances, however, 0.25 μ l of carbachol at the concentration of 0.5-1 μ g/ μ l of sterile saline were injected more caudally and ventrally in large-celled areas of the brainstem RF (usually at P5, L1.5, H-6.5). Placement of the cannula, drug delivery, and histological localization of the tip of the cannula as well as of the injection area were performed following the methods described in a previous paper (8). Finally, in one experiment unilateral injection of carbachol into the dorsal aspect of the pRF was performed 40 days after kainic acid destruction of the gigantocellular tegmental field (FTG) of the same side. In this instance 0.5 μ l of a kainic acid solution, at the concentration of 2.5 μ g/ μ l, were injected under Nembutal anesthesia at the stereotaxic coordinates of P5, L1.5, H-5.0, using a needle inclined 30° from the vertical. After infusion of the cholinergic agonist in the appropriate brainstem structures, the muscarinic receptor antagonist atropine sulphate was injected either locally in the dorsal aspect of the pontine tegmentum (0.25 μ l at the concentration of 6-60 μ g/ μ l of sterile saline) or intravenously at the dose of 0.25 mg/kg. Some of the experiments referred to above had been used in a previous paper (8) to document the increased gain of the vestibulospinal reflexes following microinjections of small doses of cholinergic agonists in the pRF.

RESULTS

1. *Episodes of postural atonia following local injection of the cholinergic agonist carbachol in the dorsal aspect of the pontine reticular formation.*

Local injection of 0.05-0.25 μ l of the cholinergic agonist carbachol (at the concentration of 0.01-0.2 μ g/ μ l of saline) performed in the dorsolateral pontine tegmentum 2-4 hours after decerebration produced a great reduction and even the abolition of the decerebrate rigidity. Simultaneously there was a great reduction and even the suppression of the spontaneous EMG activity recorded from the forelimb extensor triceps brachii. Even the myotatic reflex, elicited by an increase in the static stretch of that muscle, was suppressed following injection of the cholinergic agonist. These effects occurred mainly ipsilaterally to the side of the injection, but they could extend also to the contralateral side.

Fig. 1 shows the suppression of the multiunit EMG activity of the left triceps brachii following microinjection of carbachol in the dorsal aspect of the ipsilateral pRF, while Fig. 2 illustrates in another experiment the abolition of the myotatic reflex of the forelimb extensor muscle ipsilaterally to the side of the injection.



Fig. 1. — Polygraphic recording of the multiunit EMG activity of the triceps brachii of both sides showing a postural asymmetry in the forelimb extensors after unilateral local injection of carbachol in the dorsal pontine reticular formation (pRF).

Precollicular decerebrate cat (Exp. 6). Bipolar records: 1: electrooculogram (EOG); 2 and 3: EMG activity of the medial head of the left and right triceps brachii, respectively. A: control taken before injection. B: 30 min after injection in the dorsal pRF of the left side (at the stereotaxic coordinates of P3, L2.8, H-3.0) of 0.05 μ l of a carbachol solution at the concentration of 0.01 μ g/ml of sterile saline, a postural atonia occurred in the forelimb extensor muscle ipsilateral to the side of the injection. This unilateral atonia was followed by an episode of bilateral atonia 20 min after a second injection of carbachol at the same dose.

In some instances the unilateral atonia was followed by a generalized bilateral suppression of postural activity after a second injection of carbachol in the same pontine region. These effects were associated with slight fluctuations, usually a slight decrease, of blood pressure and heart rate.

The postural and reflex changes induced by carbachol injections appeared 10-30 min after administration of carbachol and continued in some instances for 30-120 min in the absence of external stimuli.

The sites which upon injection produced the postural changes described above were located in the dorsal aspect of the pRF immediately ventral to the LC. Injections in more ventrally located areas were less effective. Fig. 3 illustrates the localization of the tip of the cannula used to inject the cholinergic agonist in two representative experiments, while Fig. 4 summarizes on a schematic sagittal section of the brainstem the points, obtained from different experiments, which produced the episodes of postural atonia following local injection of carbachol. It is clear from this figure that most of the effective points were located in the peri-LC α as well as in the surrounding pRF.

In most of these experiments no oculomotor activity occurred spontaneously following cholinergic activation of these pontine structures. In one experiment (Exp. 2), however, the postural atonia which affected the left forelimb ipsilateral

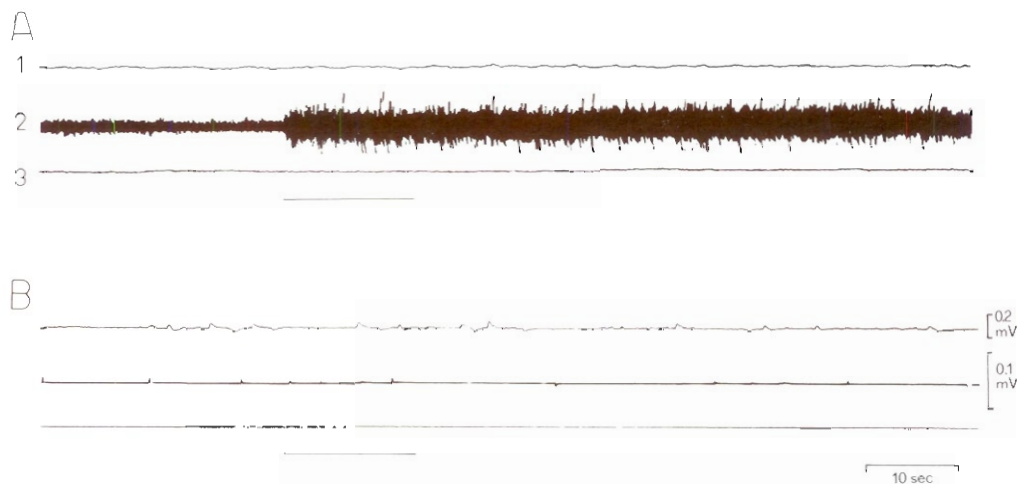


Fig. 2. — Effects of unilateral local injection in the pRF of carbachol on the response of the ipsilateral triceps brachii to static stretch.

Precollicular decerebrate cat (Exp. 4). Bipolar records: 1: EOG; 2: EMG activity of the medial head of the left triceps brachii. The horizontal lines indicate the duration of the passive flexion of the left forelimb leading to static stretch of the corresponding triceps brachii.

A: record taken 10 min after injection of 0.1 μ l of a carbachol solution at the concentration of 0.02 μ g/ μ l of sterile saline in the dorsal pRF (at P3, L2.8, H-2.5), leading to an increased gain of the vestibulospinal reflex acting on the ipsilateral triceps brachii. Notice the multiunit EMG response of the left triceps brachii to muscle stretch which outlasts the duration of the stimulus.

B: 77 min after the injection of carbachol, there was an episode of postural atonia characterized by suppression not only of the spontaneous EMG activity but also of the response of the left triceps brachii to muscle stretch. Similarly the vestibulospinal reflex was abolished (not shown).

to the side of the injection (0.25 μ g of carbachol solution at the concentration of 0.2 μ g/ μ l of saline) was followed after 30 min by a nystagmus, the fast component of which was oriented towards the side of the injection (Fig. 5). This nystagmus could be attributed to some asymmetric activity in the pontine tegmental structures of both sides; however, superimposed on this unidirectional nystagmus, bursts of REM occurred at regular intervals of one every 4-5 sec. This finding is illustrated in Fig. 6; note that, as soon as this autochthonous rhythmic activity appeared, the number of rapid ocular movements which characterized each burst progressively increased from one jerk, as observed 7 min after the appearance of the nystagmus (A), to 3-4 jerks as observed 25 min after the nystagmus (D). This pattern of oculomotor activity, which was not associated with tonic or phasic changes in blood pressure and heart rate (Fig. 6), continued spontaneously for about 40 min in absence of external stimuli. At the end of this episode both the spontaneous nystagmus as well as the superimposed rhythmic oculomotor activity disappeared; yet the postural atonia was still present in the ipsilateral forelimb, until the decerebrate rigidity as well as the spontaneous EMG activity reappeared in the corresponding triceps brachii.

Arousing stimuli, such as acoustic or somatosensory stimuli (pressure applied

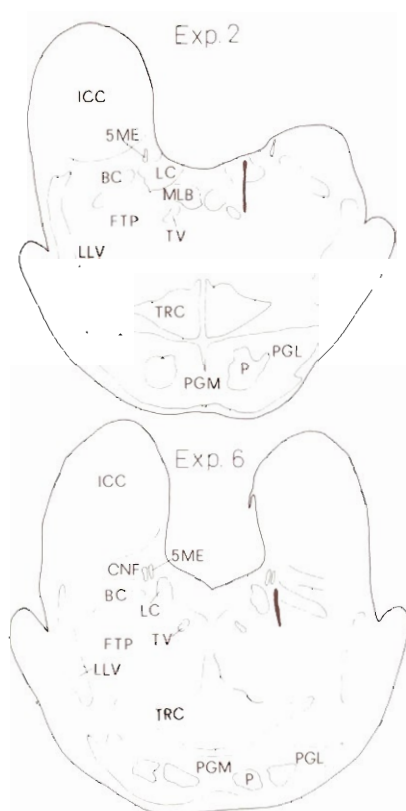


Fig. 3. — Schematic representation of transverse sections of the pons showing in two experiments the localization within the dorsal pRF of the tip of the needle at which injection of carbachol produced postural atonia as well as suppression of the multiunit EMG responses of the ipsilateral triceps brachii to labyrinth stimulation.

Precollicular decerebrate cats (Exps. 2 and 6). In both experiments the site of the injections corresponds to the dorsal aspect of the pRF located either ventromedially or laterally to the locus coeruleus. In particular, carbachol was injected at the following concentrations: 0.25 μ l at 0.2 μ g/ μ l in Exp. 2 and 0.1 μ l at 0.01 μ g/ μ l repeated twice in Exp. 6. All these injections abolished the postural activity and suppressed the multiunit EMG response of the ipsilateral triceps brachii to animal tilt (at 0.15 Hz, $\pm 10^\circ$).

Abbreviations: BC, brachium conjunctivum; CNF, cuneiform nucleus; FTP, paralemniscal tegmental field; ICC, central nucleus of the inferior colliculus; LC, locus coeruleus; LLV, ventral nucleus of the lateral lemniscus; MLB, medial longitudinal bundle; P, pyramidal tract; PGL, pontine gray, lateral division; PGM, pontine gray, medial division; TPC, tegmental reticular nucleus, central division; TV, ventral tegmental nucleus; 5ME, mesencephalic trigeminal nucleus.

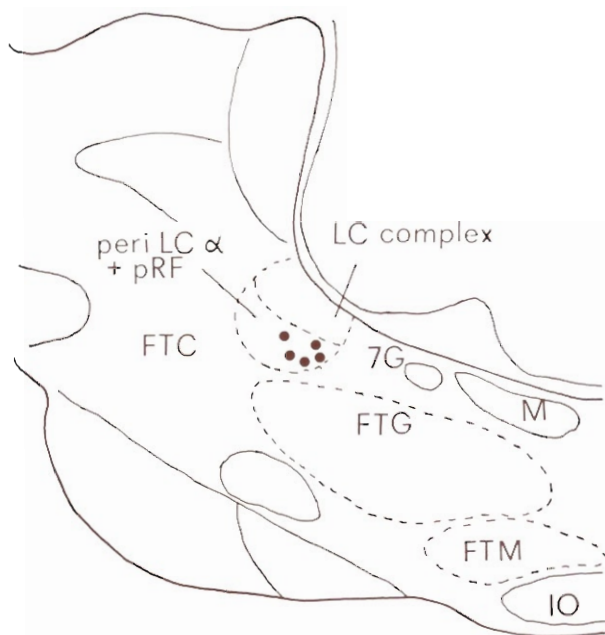


Fig. 4. — Schematic sagittal section of the brain stem showing the points which, upon injection of carbachol at the appropriate concentration, produced a postural atonia and suppressed the multiunit EMG responses of the ipsilateral triceps brachii to roll tilt of the animal.

The brain stem corresponds to Berman's sagittal plane 2.5. There were a total of 5 injection sites within the dorsal pRF in three precollicular decerebrate cats (Exps. 2,3 and 6). The dose which upon injection suppressed the postural activity as well as the EMG modulation of the ipsilateral triceps brachii to animal tilt (at 0.15 Hz, $\pm 10^\circ$) corresponded to 0.05-0.25 μ l of a carbachol solution of 0.01-0.2 μ g/ μ l of sterile saline. The injection sites were identified by adding pontamine 5% to saline.

Abbreviations: FTC, central tegmental field; FTG, gigantocellular tegmental field; FTM, magnocellular tegmental field; IO, inferior olive; LC, locus coeruleus; M, medial vestibular nucleus; peri LC α , peri-locus coeruleus alpha; pRF, dorsal pontine reticular formation; 7G, genu of the facial nerve.

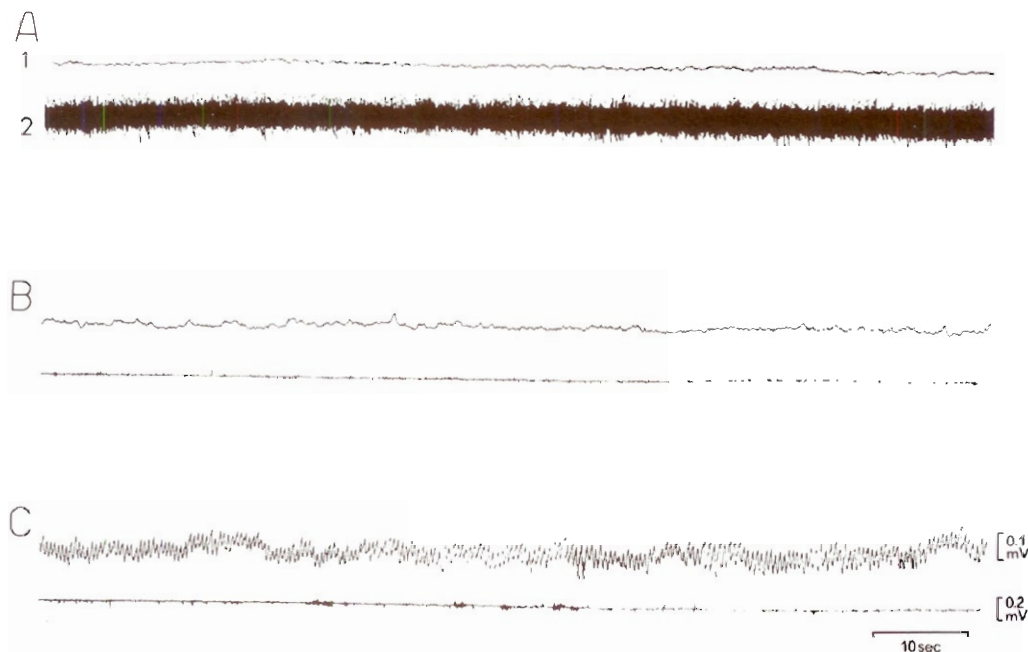


Fig. 5. — *Spontaneous nystagmus during an episode of postural atonia produced by unilateral local injection of carbachol in the dorsal aspect of the pRF.*

Precollicular decerebrate cat (Exp. 2). Bipolar records: 1: EOG; 2: EMG activity of the medial head of the left triceps brachii; A: control records taken before injection. B: 3 min after injection in the dorsal aspect of the pontine tegmentum of the left side (at the stereotaxic coordinates of P3,L2.8, H-2.0) of 0.25 μ l of a carbachol solution at the concentration of 0.2 μ g/ μ l of sterile saline a postural atonia occurs in the forelimb extensor muscle ipsilateral to the side of the lesion. This postural change was not associated with oculomotor activity. C: 33 min after the injection a spontaneous nystagmus appears, characterized by slow eye movements towards the right side followed by fast movements towards the left side (downward deflection in the EOG).

to limb extremities), which in normal decerebrate cats produced a generalized increase in postural activity following active flexion of the stimulated limb, did not suppress the postural atonia nor did they interfere with the occurrence of both the spontaneous nystagmus and the superimposed rhythmic oculomotor activity.

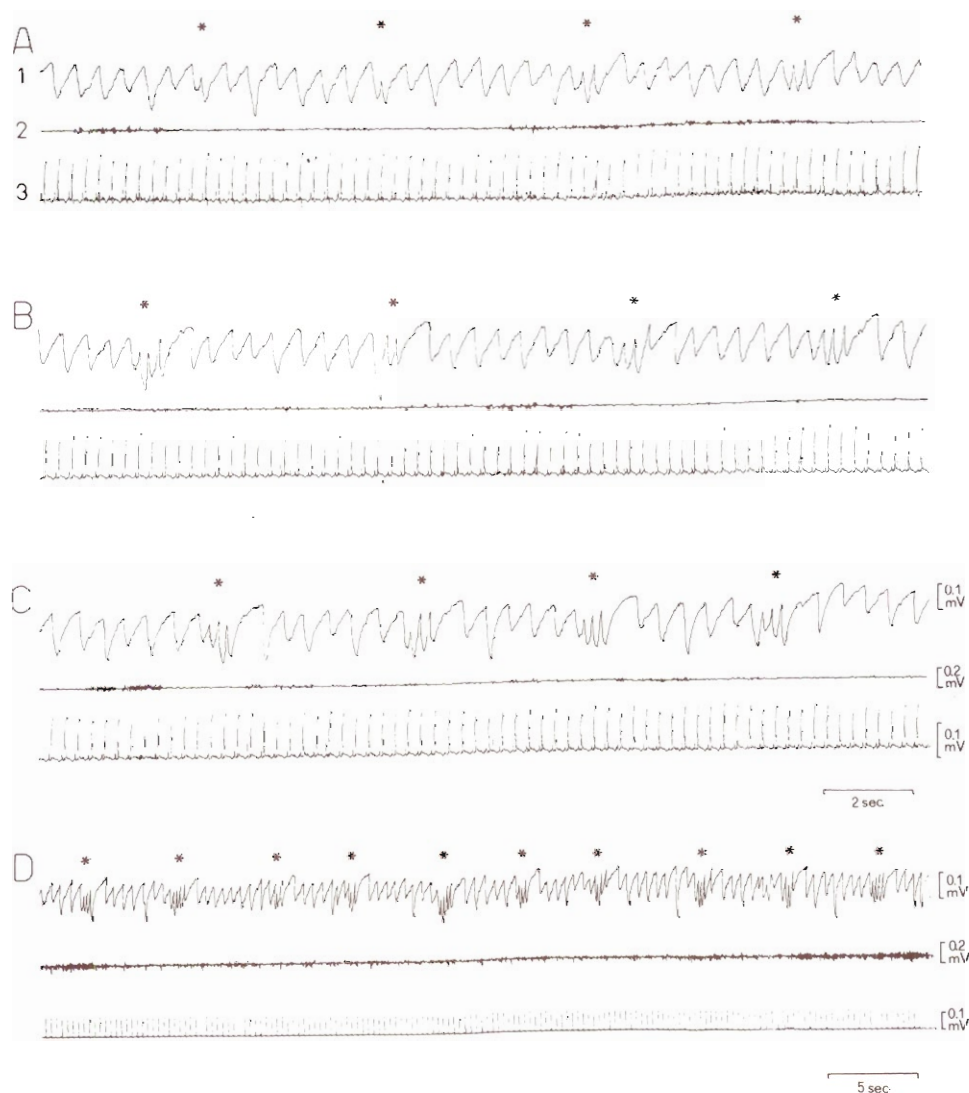


Fig. 6. Bursts of rapid eye movements (REM) superimposed on a background of spontaneous nystagmus during an episode of postural atonia following unilateral local injection of carbachol in the dorsal aspect of the pRF.

Same experiment as in Fig. 5. Bipolar records. 1: EOG; 2: EMG of the medial head of the left triceps brachii; 3: electrocardiogram (EKG). The records were taken 37 min (A), 43 min (B), 50 min (C) and 55 min (D) after local injection in the dorsal pRF of the left side of 0.25 μ l of a carbachol solution at the concentration of 0.2 μ g/ μ l of sterile saline. During the unidirectional nystagmus characterized by fast eye movements towards the left side (downward deflection in the EOG), bursts of REM occurred at regular intervals of one burst every 4-5 sec, as indicated by asterisks. Notice the progressive increase of rapid eye movements within each burst during the development of this rhythmic oculomotor activity.

2. Inhibition of vestibulospinal reflexes following local injection of carbachol in the dorsal aspect of the pontine reticular formation.

In all the decerebrate cats tested prior to carbachol injections, a sinusoidal modulation of the multiunit EMG activity of the triceps brachii was observed during roll tilt of the animal at the frequency of 0.15 Hz, $\pm 10^\circ$. In particular, the first harmonic component of the EMG responses to labyrinth stimulation, which represented the predominant component of the responses, was characterized by an increased activity during side-down tilt of the animal and a decreased activity during side-up tilt (α -responses) (cf. 43). These responses were completely suppressed during the episodes of postural atonia induced by carbachol administration.

Fig. 7 illustrates the modulation of the EMG activity of the triceps brachii

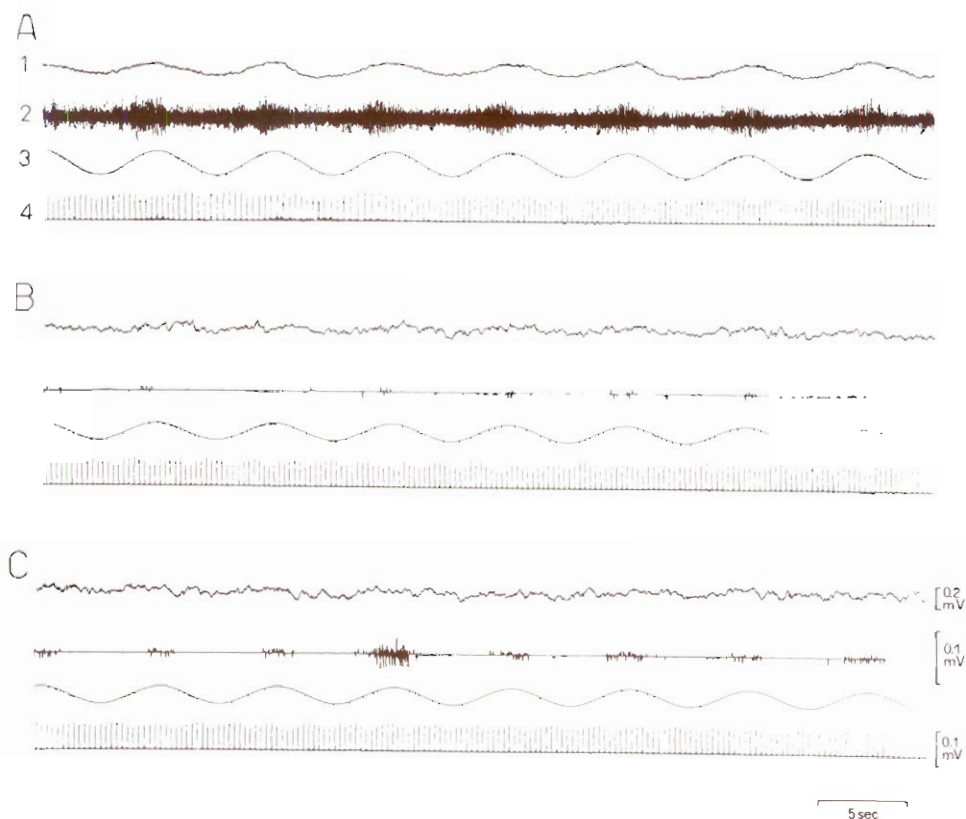


Fig. 7. — Suppression of the multiunit EMG response of the left triceps brachii to roll tilt of the animal after injection of carbachol in the ipsilateral dorsal pRF.

Same experiment as in Fig. 5. Bipolar records: 1: EOG; 2: EMG of the medial head of the left triceps brachii; 3: animal position; 4: EKG. A: control records taken before injection showing a sinusoidal modulation of the multiunit EMG activity of the left triceps brachii during roll tilt of the animal at 0.15 Hz, $\pm 10^\circ$ (ipsilateral side-down tilt of the animal is indicated by an upward deflection in the sine wave). The EMG modulation is associated with the appearance of a compensatory vestibulo-ocular reflex.

B and C: great depression or suppression of the vestibulospinal and the vestibulo-ocular reflexes during the episode of postural atonia induced by the cholinergic agonist.

of one side elicited by animal tilt in a decerebrate cat. In particular, an increased muscular activity occurred during ipsilateral side-down animal displacement; moreover, the responses were related to animal position and not to the velocity of animal displacement (A). Local injection of carbachol in the pRF, which abolished the postural rigidity as well as the spontaneous EMG activity of the ipsilateral triceps brachii, also suppressed the modulation of the multiunit EMG activity of the same muscle during animal tilt (8). During the episode of postural atonia some slight unit activity occurred from time to time, which was apparently related to the extreme side-down animal tilt (C); these episodes, however, were short-lasting and were soon followed by a complete suppression of the EMG modulation during tilt¹.

It is of interest that when the dose of carbachol was rather small, the suppression of the spontaneous postural activity as well as of the EMG response of the triceps brachii to animal tilt was preceded by episodes of postural hypotonia, characterized by an increased amplitude of the EMG modulation during animal tilt. The conditions which led to the appearance of these episodes were reported in a previous study (8). However, when the postural atonia occurred a few minutes after local injection of the cholinergic agonist carbachol, the amplitude of the EMG modulation during tilt suddenly decreased, until the responses were suppressed. This finding paralleled the great reduction or the abolition of the spontaneous EMG activity of the triceps muscle which occurred after injection of the cholinergic agonist.

3. *Effects of injection of the muscarinic receptor antagonist atropine sulphate on the episodes of postural atonia and related inhibition of vestibulospinal reflexes following local injection of carbachol in the dorsal aspect of the pontine reticular formation.*

The episodes of postural atonia as well as the suppression of the vestibulospinal reflexes described in the previous sections were substituted by a good decerebrate rigidity and by the reappearance of these proprioceptive reflexes following injection in the dorsal aspect of the pRF of small doses of atropine sulphate. In particular, local injection of 0.25 μ l of this muscarinic receptor blocker, at the concentration of 6-60 μ g/ μ l of sterile saline, suppressed the postural atonia, while the gain of the EMG responses of the triceps brachii to labyrinth stimulation returned to the control values or reached even higher levels.

In contrast to these findings, intravenous injection of atropine sulphate at a dose of 0.25 mg/kg did neither suppress the postural atonia induced by local injection of carbachol in the dorsal pRF nor did it produce the recovery of both the spontaneous EMG activity as well as the EMG modulation of the triceps brachii to labyrinth stimulation. There is evidence, however, that systemic injection of atropine at the dose indicated above partially counteracted the effects of carbachol. In fact, an increased static stretch of the muscle following passive flexion

¹ Fig. 7 is of interest since it shows that even the vestibulo-ocular reflex, which was surprisingly present in this decerebrate cat (A), was severely impaired during the induced episode of postural atonia (B and C).

of the forelimb, which was unable by itself to produce a spontaneous or induced EMG activity in the corresponding triceps brachii during animal tilt after carbachol injection, now brought to the light a clear-cut EMG modulation of the triceps brachii, which was more prominent than that obtained in the control situation for the same parameters of labyrinth stimulation and actually outlasted the duration of the forelimb flexion (Fig. 8).

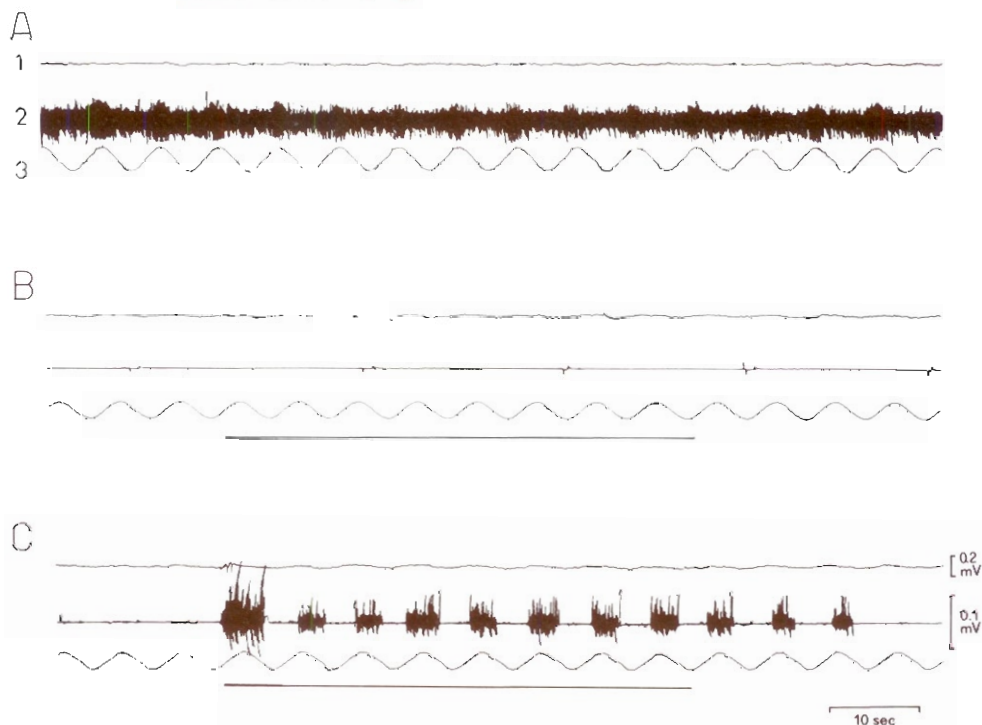


Fig. 8. — Polygraphic recording of the multiunit EMG activity of the left triceps brachii showing the suppression of its response to roll tilt of the animal after local injection of carbachol in the ipsilateral dorsal pRF leading to postural atonia and its recovery after systemic injection of a muscarinic blocker.

Same experiment as in Fig. 2. In each group of records, the upper trace represents the EOG (1), the middle trace the EMG activity of the medial head of the left triceps brachii (2), the lower trace the animal displacement. The animal was submitted to roll tilt at the standard parameters of 0.15 Hz, $\pm 10^\circ$; slight differences in amplitude of the sine wave in A-C are due to changed gain in the polygraphic records. Ipsilateral side-down tilt of the animals is indicated by upward deflection in the sine wave.

A: multiunit EMG activity recorded during roll tilt of the animal 5 min after injection of 0.1 μ l of a carbachol solution at the concentration of 0.02 μ g/ μ l of sterile saline in the ipsilateral dorsal pRF (at P3, L2.8, H-2.5). The EMG activity of the left triceps brachii increased periodically during ipsilateral side-down tilt of the animal; the amount of the modulation and thus the response gain of the vestibulospinal reflex acting on the ipsilateral triceps brachii were higher than those obtained prior to the injection.

B: record taken 70 min after injection of carbachol, during an episode of postural atonia characterized by a suppression not only of the spontaneous EMG activity, but also of the response of the triceps brachii to sinusoidal animal tilt. The EMG modulation during animal tilt was not restored even by increasing the static stretch of the muscle (horizontal line).

C: two intravenous injections of 0.25 mg/kg of atropine sulphate were performed 2 hours and 2 hours and 10 min after carbachol injection. Records taken 21 min after the second injection of the muscarinic blocker. There was neither a recovery in the spontaneous EMG activity nor in the responses of the triceps brachii to animal tilt at the same parameters reported above. However, an increased static stretch of the muscle following passive flexion of the forelimb (horizontal line) brought to the light a clear-cut EMG modulation which outlasted the duration of the forelimb flexion.

The averaged multiunit responses of the triceps brachii to labyrinth stimulation in the three experimental conditions illustrated in the previous figure, *i.e.* in the control situation, after local injection of carbachol in the ipsilateral pRF, as well as after systemic injection of atropine sulphate during static stretch of the muscle, are illustrated in Fig. 9 A, B and C, respectively. In particular, it appears that

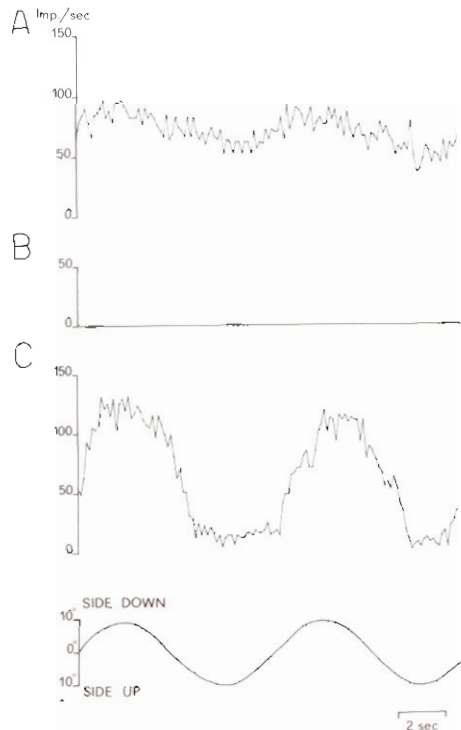


Fig. 9. — *Suppression of the multiunit EMG response of the triceps brachii to roll tilt of the animal after local injection of carbachol in the dorsal pRF leading to postural atonia and its recovery after systemic injection of a muscarinic blocker.*

Same experiment as in Fig. 8. Sequential pulse density histograms (SPDHs) showing the average multiunit responses of the left triceps brachii to roll tilt of the animal at the frequency of 0.15 Hz, $\pm 10^\circ$ (average of 5 sweeps using 128 bins with 0.1 sec bin width). The lower trace indicates the animal displacement: ipsilateral side-down tilt of the animal is indicated by upward deflection in the sine wave.

A: multiunit EMG activity recorded during animal tilt 51 min after injection of 0.1 μ l of a carbachol solution at the concentration of 0.02 μ g/ μ l of sterile saline in the ipsilateral dorsal pRF. This record showed on the average a base frequency of 70.8 imp./sec, a gain of the first harmonic responses of 1.43 imp./sec/deg, a sensitivity of 2.02%/deg and a phase lag of -2.3° with respect to the ipsilateral side-down tilt; there was also a second harmonic whose amplitude corresponded to 33.4% of the fundamental.

B: record taken 81 min after injection of carbachol, during an episode of postural atonia induced by the cholinergic agonist. There was neither a spontaneous nor an evoked EMG activity during animal tilt at the same parameters reported above.

C: two intravenous injections of 0.25 mg/kg of atropine sulphate were performed 2 hours and 2 hours and 10 min after carbachol injection. Record taken 24 min after the second injection of the muscarinic blocker. Both the spontaneous EMG activity as well as the responses of the triceps brachii to animal tilt were still suppressed. However, an increased static stretch of the muscle following passive flexion of the forelimb produced the reappearance of the EMG modulation, whose gain was greatly increased. In particular, the average base frequency corresponded to 60.6 imp./sec, the gain was 5.52 imp./sec/deg, the sensitivity 9.10%/deg, while the phase angle corresponded to a lag of -17.3° ; interestingly, the amplitude of the second harmonic corresponded only to 0.9% of the fundamental.

the response gain of the triceps brachii to labyrinth stimulation, which corresponded to 1.43 imp./sec/deg in the control situation, decreased down to zero during the episode of postural atonia induced by the cholinergic agonist (a value which remained unmodified following static stretch of the muscle induced by passive flexion of the limb), but raised up to 5.52 imp./sec/deg following systemic injection of the muscarinic blocker atropine sulphate. In this instance, in which atropine reduced, in part at least, the excitatory influences of carbachol on the pRF neurons, a multiunit EMG response of the triceps brachii to animal tilt occurred when the excitability of the corresponding motoneurons was increased following a static stretch of the muscle.

Results similar to those elicited by passive flexion of the forelimb were also obtained by applying a gentle pressure to the pinna of the ear. In fact this stimulus, which did not elicit any spontaneous or induced EMG activity of the ipsilateral triceps brachii during animal tilt after local injection of carbachol in the corresponding dorsal pRF, produced a clear-cut modulation of the EMG activity to both a dynamic (Fig. 10A) and static (Fig. 10C) tilt of the animal after systemic injection of atropine sulphate. The effects described above appeared only when the pressure was applied to the pinna ipsilateral to the side of the EMG recording; pressure applied to the contralateral pinna was ineffective (see Fig. 10B).

4. *Episodes of postural atonia and related inhibition of vestibulospinal reflexes following local injection of carbachol in the ventral aspect of the pontine and medullary reticular formation.*

In four experiments carbachol was injected more caudally and ventrally into large-celled areas of the brainstem reticular formation. In particular, 0.25 μ l of carbachol at the concentration of 0.5-1 μ g/ μ l of saline (*i.e.*, higher than that used for the pRF) abolished both the spontaneous postural activity as well as the modulation of the multiunit EMG activity of the ipsilateral triceps brachii to sinusoidal labyrinth stimulation (Fig. 11). This effect, which in some instances involved also the limb contralateral to the side of the injection, lasted for a variable period of time until a recovery of both the spontaneous and induced EMG activity appeared. Histological controls indicated that in these experiments the tip of the cannula was located either in the FTG, or in the magnocellular tegmental field (FTM), or between the FTG and the rostral aspect of the FTM (Fig. 12).

It is of interest, however, that kainic acid destruction of the FTG of one side, performed in one experiment 40 days prior to decerebration, did neither prevent the appearance of the postural atonia nor the related suppression of the EMG responses of the triceps brachii to labyrinth stimulation following local injection of carbachol in the dorsal aspect of the ipsilateral pRF. This fact indicates that the FTG is not critical for the production of the effects described in the previous sections.

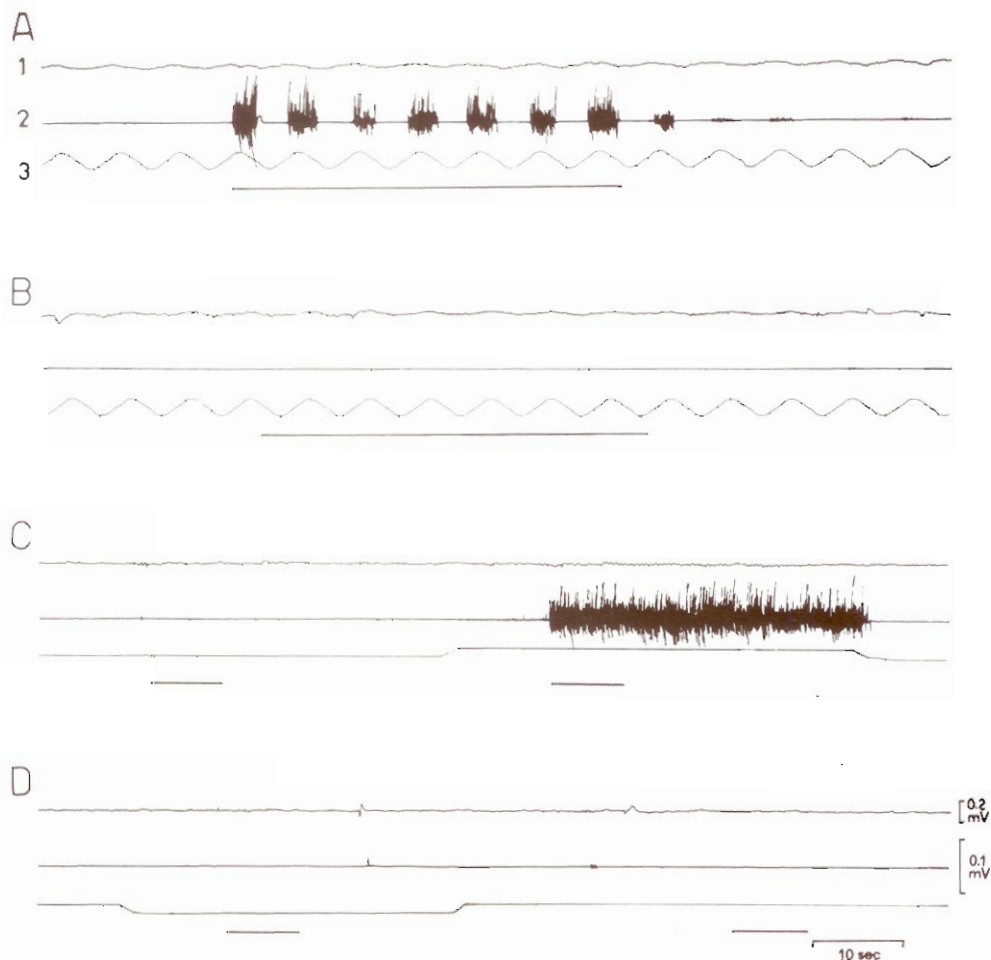


Fig. 10. — Suppression of the multiunit EMG responses of the left triceps brachii to sinusoidal or static tilt of the animal by local injection of carbachol in the ipsilateral dorsal pRF followed by systemic injection of a muscarinic blocker, and their recovery during gentle pressure applied to the pinna of the ipsilateral ear.

Same experiment as in Fig. 8, same bipolar records. Pressure applied to the pinna of the left (A, C, D) or the right ear (B) are indicated by horizontal lines. A, B: records taken 143 and 153 min, respectively, after injection of $0.1 \mu\text{l}$ of a carbachol solution at the concentration of $0.02 \mu\text{g}/\mu\text{l}$ of sterile saline in the ipsilateral dorsal pRF, followed by two injections of 0.25 mg/kg of atropine sulphate. During the induced episode of postural atonia there was a suppression not only of the spontaneous EMG activity but also of the response of the triceps brachii to roll tilt of the animal at 0.15 Hz , $\pm 10^\circ$. Gentle pressure applied to the pinna of the left ear, indicated by the horizontal line, brought to the light a clear-cut EMG modulation, which outlasted the duration of the pressure stimulation (A); the same stimulus applied to the right ear was ineffective (B).

C, D: continuous records started 7 min after B, showing absence of any spontaneous EMG activity as well as applied to the response of the triceps brachii to 10° of static tilt of the animal. Pressure applied to the left ear (indicated by horizontal lines) was ineffective in the animal at rest. The same stimulus, however, produced an increased EMG activity in the left triceps brachii during side-down tilt (upward deflection of the horizontal line in C), but not during side-up tilt of the animal (downward deflection of the horizontal line in D).

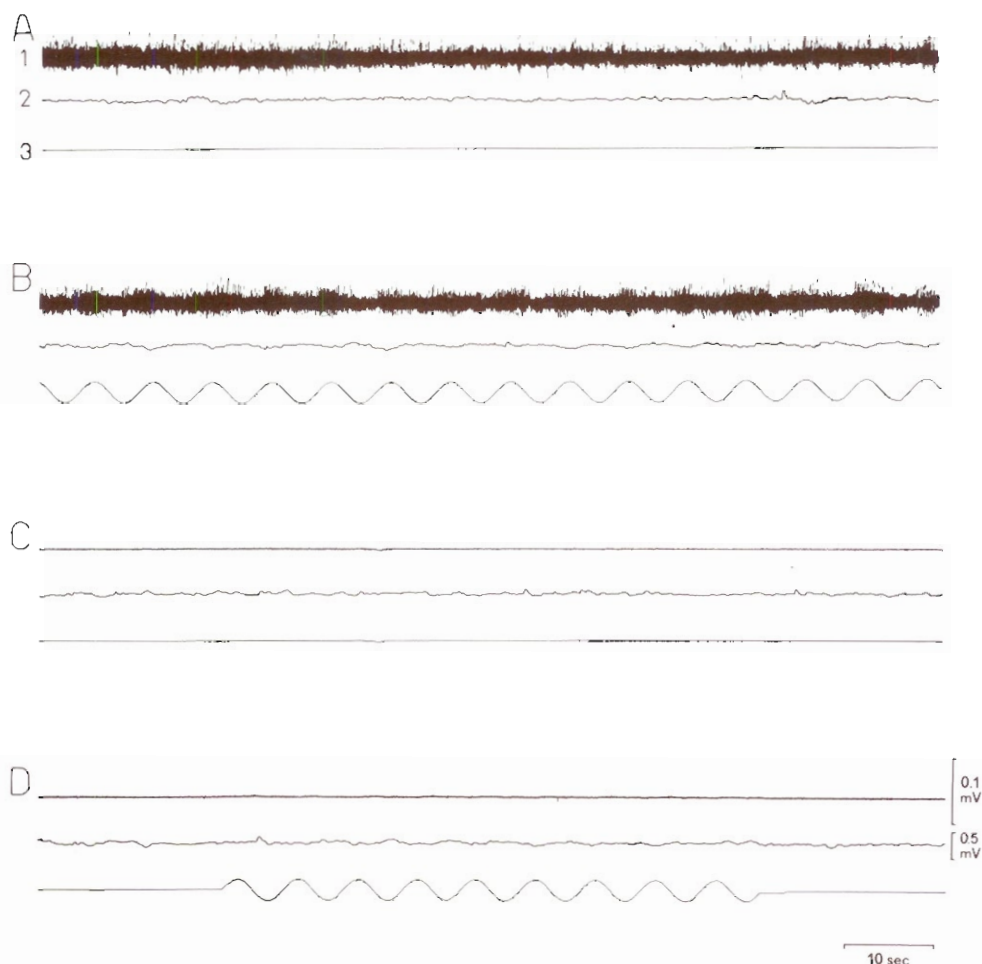


Fig. 11. — Polygraphic recording showing the suppression of the spontaneous EMG activity as well as of the responses of the triceps brachii to roll tilt of the animal after local injection of carbachol in the gigantocellular tegmental field (FTG) leading to a generalized episode of postural atonia.

Precollicular decerebrate cat (Exp. 14). Bipolar records: 1: EMG activity of the medial head of the left triceps brachii; 2: EOG; 3: animal position (ipsilateral side-down tilt of the animal is indicated by upward deflection in the sine wave). A: control taken in the decerebrate animal showing a spontaneous EMG activity in the left triceps brachii in the animal at rest; B: modulation of the EMG activity of the left triceps brachii during animal tilt (at 0.15 Hz, $\pm 10^\circ$), characterized by an increased discharge during ipsilateral side-down tilt and a reduced discharge during contralateral, side-up tilt; C: 102 sec after local injection of carbachol (0.5 μ l of carbachol at the concentration of 0.5 μ g/ μ l of sterile saline) into the FTG of the left side, showing the suppression of the spontaneous EMG activity in the left triceps brachii; D: record taken soon after C to show the suppression of the EMG responses of the triceps brachii to roll tilt of the animal at the same parameters used in B.

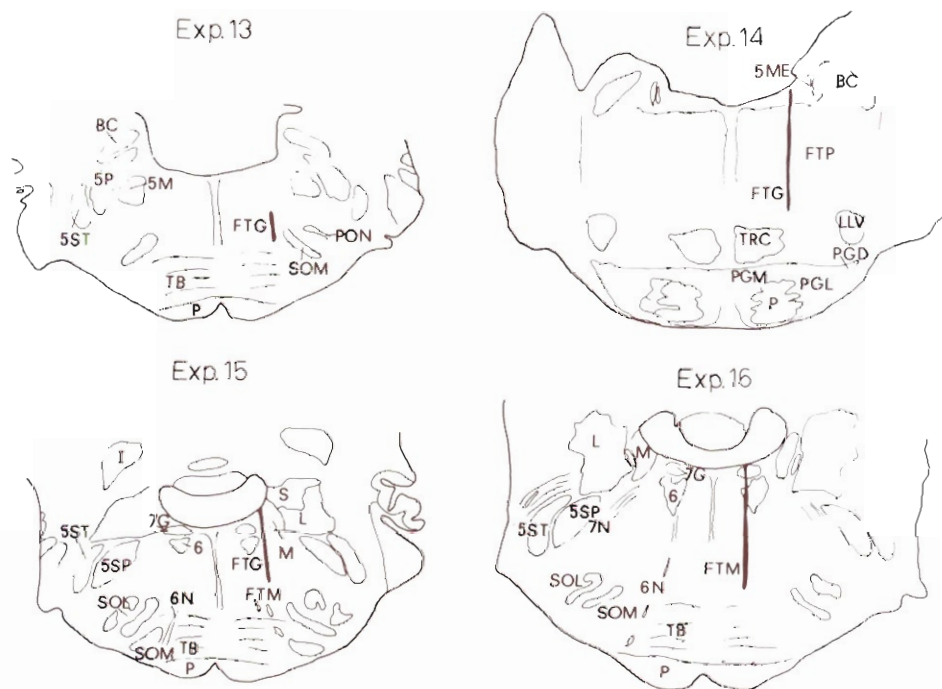


Fig. 12. — Schematic representation of transverse sections of the pons and the medulla showing in four experiments the localization within the large-celled area of the pontine and medullary reticular formation of the tip of the needle at which injection of carbachol produced postural atonia as well as suppression of the multiunit EMG responses of the ipsilateral triceps brachii to labyrinth stimulation.

The tip of the needle was located in the gigantocellular tegmental field (Exps. 13 and 14), in the medullary tegmental field (Exp. 16), or between the gigantocellular and the magnocellular tegmental field (Exp. 15).

Abbreviations: BC, brachium conjunctivum; FTG, gigantocellular tegmental field; FTM, magnocellular tegmental field; FTP, paralemniscal tegmental field; I, interpositus nucleus; L, lateral vestibular nucleus; LLV, ventral nucleus of the lateral lemniscus; M, medial vestibular nucleus; P, pyramidal tract; PGD, pontine gray, dorsolateral division; PGL, pontine gray, lateral division; PGM, pontine gray, medial division; PON, preolivary nucleus; S, superior vestibular nucleus; SOL, lateral nucleus of the superior olive; SOM, medial nucleus of the superior olive; TB, trapezoid body; TRC, tegmental reticular nucleus, central division; 5M, motor nucleus of the trigeminal nerve; 5ME, mesencephalic nucleus of the trigeminal nerve; 5P, principal sensory trigeminal nucleus; 5SP, spinal trigeminal nucleus, parvocellular division; 5ST, spinal tract of the trigeminal nerve; 6, abducens nucleus; 6N, abducens nerve; 7G, genu of the facial nerve; 7N, facial nerve.

DISCUSSION

The present experiments have shown that local injection of the cholinergic agonist carbachol into the dorsal pontine tegmentum suppressed not only the decerebrate rigidity but also the myotatic reflexes as well as the vestibulospinal reflexes. At the doses used in these experiments the effects involved mainly the limbs ipsilateral to the side of the injection; in some instances, however, contralateral limbs could also be affected. The effects were site-specific, since they were particularly obtained when the substance was injected in reticular structures located immediately ventral to the LC, namely in the peri-LC α and the surrounding pRF. Moreover, they were reversible but could be prolonged by a successive injection of the same dose of carbachol.

The *first* conclusion to be drawn from this study is that local pontine infusion of a cholinergic agonist produced postural and reflex changes fundamentally similar to those obtained in decerebrate cats after systemic administration of the anti-AChE eserine sulphate (at least 0.10 mg/kg) (44). It is known that eserine acts as an AChE inhibitor, thereby producing accumulation of ACh at those places where it is released. It is reasonable, therefore, to assume that the cholinceptive neurons activated by systemic injection of the anti-AChE correspond to those identified in the present experiments following local injection of carbachol. There are nevertheless some understandable differences, which may be summarized as follows.

While in the experiments with the anti-AChE postural and reflex changes involved the limb extensor musculature of both sides (44), in the present experiments the effects were more prominent ipsilaterally to the injection site. This difference can easily be explained, since in the former experiments the anti-AChE was injected systemically, thus involving cholinceptive structures of both sides, while in the present experiments the cholinergic agonist was injected locally in the dorsal pontine tegmentum of one side.

A further difference was that, while the episodes of postural atonia induced by the anti-AChE were associated with the typical occurrence of REM bursts (44; cf. also 40, 45, 46, 50), this rhythmic oculomotor activity was barely observed after local injection of carbachol. This finding indicates that the cholinceptive structures involved in the production of REM bursts are at least in part different from those which are critical for postural atonia, and that only the latter were involved by carbachol injections as described in this study.

A final difference was that, while in previous experiments (44) arousing stimuli (e.g., somatosensory, nociceptive or acoustic stimuli) suppressed the episodes of postural atonia induced by the anti-AChE, thus leading to the reappearance of decerebrate rigidity as well as of EMG responses of forelimb extensor muscles to labyrinth stimulation, in the present experiments the same stimuli failed to do so. It is likely that the direct activation of cholinceptive neurons in the dorsal pontine tegmentum, produced by local injection of carbachol, was so prominent to prevent that the animal state was modified by the arousing stimuli.

The *second* point to be stressed regards the exact localization of the region which is critically responsible for the effects described above. Histologic examination of the effective injection sites indicated that this region corresponded to the peri-LC α and the surrounding pRF. This conclusion is supported by the results of previous lesion and single unit recording experiments indicating that the peri-LC α and the neighbouring pRF are critically implicated in the generation of postural atonia during D (66, 68, see also below). However, according to some Authors, experiments of unit recording performed during D in head-restrained cats (27, 28, 47-49) as well as experiments of chemical stimulation leading to the occurrence of D in intact cats (1, 75, 82) indicated that the pontine structure involved in the generation of postural atonia was the FTG. In spite of that, neurochemical lesion experiments performed in intact animals (71) as well as in decerebrate cats tested with an anti-AChE (8) showed that destruction of the FTG has no effect on the appearance of postural atonia. Furthermore, it should be mentioned that the discharge pattern of FTG neurons, which is phasic and extended to active wakefulness (74), is not well suited for the generation of postural atonia. The present experiments lend further support to the conclusion that the FTG is not essential to the induction of postural atonia. In fact, even if episodes of postural atonia could be induced by injection of carbachol in the FTG, kainic acid destruction of the FTG of one side did not prevent the appearance of these episodes following microinjection of carbachol in the peri-LC α and the surrounding dorsal pRF. This finding can easily be explained, since kainic acid destroyed selectively only the cell bodies but not the fibers which, originating from the dorsal pRF, cross the FTG before reaching the magnocellular area of the medullary RF, from which the inhibitory reticulospinal system originates.

It would be of interest to know the precise nature of the receptors on which the cholinomimetic substance operated in our experiments, since carbachol is a cholinergic agonist acting on both muscarinic and nicotinic receptors. Our findings have shown that local injection of the muscarinic receptor antagonist atropine in the same pontine area as carbachol produced the reappearance of the decerebrate rigidity as well as of the modulation of multiunit EMG activity of the triceps brachii to labyrinth stimulation. Similar results were obtained in intact animals, where atropine pretreatment completely blocked the episodes of postural atonia (associated with D) which normally follow local pontine injection of carbachol (4) or neostigmine (3, 6). Moreover, atropine pretreatment completely blocked the episodes of postural atonia induced by i.v. injection of an anti-AChE (40, 46). Since atropine blocks the muscarinic effects, this would support the hypothesis that the episodes of postural atonia described above were mediated via muscarinic receptors. This conclusion is strengthened by the fact that episodes of postural atonia associated with D could also be elicited, in intact animals, by pontine injection of pure muscarinic agonists like oxytremorin (17) or betanecol (25).

On the other hand, it should be noticed that, in the present experiments, systemic injection of atropine was much less effective than local injection in reverting the postural and reflex changes induced by carbachol infusion into the dorsal pontine

tegmentum, since it was not able to suppress the postural atonia nor to produce the recovery of the EMG modulation of the triceps brachii to labyrinth stimulation. However, after i. v. injection of atropine sulphate, a tonic increase in static stretch of forelimb extensor muscles or a steady gentle pressure applied to the pinna of the ear, which are known to increase muscle spindle discharge, but were ineffective after the injection of carbachol, produced the reappearance of both the myotatic and the pinna reflexes and led to a prominent modulation of EMG activity of the triceps brachii to sinusoidal labyrinth stimulation. These findings indicate that systemic injection of the muscarinic blocker may only partially antagonize the effects of direct activation of the cholinceptive neurons located in the pontine reticular structures following local injection of carbachol.

Let us examine now the mechanisms responsible for the postural and reflex changes described above. If we accept the analogy between postural atonia induced by the injection of carbachol into the pontine tegmental structures in the decerebrate cat and that occurring spontaneously during D in the intact animal, we should consider the large body of evidences suggesting that activation of cholinceptive cells located in the dorsal pontine tegmentum tonically excites a magnocellular region of the mRF, which is in turn able to powerfully inhibit α -motoneurons. In fact: i) dorsal pontine tegmental structures contain many tonic units which fire selectively during the episodes of postural atonia occurring spontaneously during D (66, 68) or induced by an anti-AChE in the decerebrate cat (29; cf. 65); ii) electrolytic or chemical destruction of this region abolished the atonia of D (22, 70); iii) the dorsal pontine tegmentum contains cholinceptive (10, 11, 21, 56, 73, 83) and/or cholinergic cells (35-37, 57-59); iv) injection of minute amounts of cholinergic agonists in this pontine area of intact, unanesthetized cats can produce episodes of postural atonia (17, 34, 51, 75, 79, 80); v) the same reticular region projects directly to the magnocellular area of the mRF (31, 69), from which the inhibitory reticulospinal pathway originates (30, 38, 60; cf. 42); moreover, many units located in this medullary reticular area were depolarized or fired selectively during the episodes of postural atonia of D (15, 33, 66, 68); finally vi) intracellular recording studies have shown that α -motoneurons are postsynaptically inhibited during the episodes of postural atonia of D (14, 18-20, 52-54).

Likewise, the suppression of proprioceptive, myotatic and vestibulospinal reflexes can be attributed to the same mechanism operating during the episodes of postural atonia produced by the anti-AChE in decerebrate cats (72; cf. 64, 65). Classical decerebrate rigidity depends on supraspinal excitatory influences on extensor motoneurons originating in the lateral vestibular nuclei (39) and the lateral reticular formation (42). In contrast, the magnocellular region of the mRF, which exerts a postsynaptic inhibitory influence on both extensor and flexor motoneurons, is apparently inactive in the decerebrate preparation (42). Experiments performed in precollicular decerebrate cats have shown that the extensor monosynaptic reflexes in the hindlimb, which were severely depressed or abolished during the episodes of postural atonia induced by the anti-AChE, were restored to their previous amplitude after

complete section of the spinal cord at T₁₂ (72). Since this operation interrupts all descending facilitatory pathways, the great depression of the monosynaptic reflexes induced by the anti-AChE prior to postbrachial section of the spinal cord could not be attributed to abolition of excitatory volleys. Moreover, experiments of unit recording have shown that the firing rate of lateral vestibular neurons was not modified during the episodes of atonia following the systemic injection of an anti-AChE (78). On the other hand, the anti-AChE tonically increased the activity of the reticulospinal system which is responsible for the inhibitory component of the spino-bulbo-spinal reflex (7, 9, 24, 41, 72, 77; cf. 64 for ref.). This system originates from the magnocellular part of the mRF (12) and courses along the ventral quadrants of the spinal cord, where the reticulospinal pathways are located (41). More direct evidence comes from recent experiments showing that neurons located in the dorsal aspect of the pRF (29; cf. 65) as well as presumably inhibitory reticulospinal neurons, located in the medial aspects of the mRF (76), undergo a state-dependent behavior, in that they increase their firing rate during the episodes of postural atonia induced by an anti-AChE. It was postulated, therefore, that the depression of the spinal reflexes induced by the anti-AChE was not due to reduction of tonic facilitatory influences mediated by the lateral vestibulospinal system, but to active inhibitory influences exerted by reticulospinal neurons on the spinal cord. In agreement with these conclusions are the results of recent experiments showing that, in the decerebrate cat, the suppression of motor activity which occurs following injection of carbachol into the pRF was due to postsynaptic inhibition of α -motoneurons (55).

SUMMARY

1. The multiunit EMG activity of the forelimb extensor muscle triceps brachii was recorded in precollicular decerebrate cats, either at rest or during roll tilt of the animal at 0.15Hz, $\pm 10^\circ$ leading to sinusoidal stimulation of labyrinth receptors. Both the spontaneous EMG activity as well as the labyrinthine-induced EMG responses were tested before and after pontine microinjection of a cholinergic agonist.

2. Local injection of the cholinergic agonist carbachol into the dorsal aspect of the pontine tegmentum (usually 0.25 μ l, 0.01-0.2 μ g/ μ l) produced a state of postural atonia, and abolished both the spontaneous EMG activity as well as the EMG responses of the triceps brachii to sinusoidal stimulation of labyrinth receptors. This suppression was generally ipsilateral to the side of the injection and persisted throughout the episode of postural atonia, but sometimes it involved also the contralateral limbs. In these instances it could be accompanied by a spontaneous nystagmus, interspersed at regular intervals with bursts of rapid eye movements.

3. Similar effects were also obtained following injection of carbachol in the gigantocellular tegmental field (FTG) (0.25 μ l, 0.5-1.0 μ g/ μ l). However, this struc-

ture was not critically responsible for the phenomena reported above, which persisted unaltered after kainic acid lesion of the FTG performed ipsilaterally to the side of the pontine injection.

4. Local infusion of the muscarinic blocker atropine sulphate reversed the effects of carbachol injection into the dorsal aspect of the pontine tegmentum, thus indicating that muscarinic receptors were involved.

5. It is postulated that the postural atonia as well as the tonic depression of vestibulospinal reflexes, which occur in the decerebrate cat after local injection of a cholinergic agonist depends, at least in part, on the activation of cholinceptive neurons located in dorsal pontine reticular structures. These may in turn excite medullary reticulospinal neurons, which are finally responsible for the inhibition of extensor motoneurons.

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