ROLE OF CATECHOLAMINES IN THE CONTROL OF THE SLEEP-WAKING CYCLE

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THE STUDY of the role of catecholamine (CA) containing neurons of the central nervous system of the cat is difficult: the pharmacological approach (inhibition of synthesis of CA or injection of precursor) is easy, but it lacks the topographical dimension which is of paramount importance since histochemical methods have shown that the CA containing neurons belong to different anatomical systems (DAHLSTRÖM and FUXE, 1964). Since the topography of CA containing neurons is now relatively well known in the cat (MAEDA *et al.*, 1973), a direct approach upon these systems is possible. This can be done by coagulation. But this method is not selective enough since other neurons may be destroyed by the lesion. Recently, 6-hydroxydopamine (6-OHDA) has appeared to be a very selective tool for destroying CA containing neurons. However, our results suggest that 6-OHDA is not as selective for CA neurons in the cat as it is in the rat.

We shall restrict this review to the neuropharmacological, neurophysiological and biochemical approaches for studying some groups of CA containing neurons in the regulation of the sleep-waking cycle in the cat. All the experiments were made in chronically implanted cats, polygraphically recorded 23 hr a day.

ANATOMICAL ORGANISATION OF THE CA NEURONS IN THE CAT BRAIN STEM

Two main ascending pathways originating from the pontine group of CA neurons (nucleus locus coeruleus, subcoeruleus, parabrachialis) which correspond to groups A_6 A_7 of the rat have been mapped out by MAEDA *et al.* (1973): the ascending dorsal CA bundle originates from the perikarya of the dorsal group of CA neurons of the pons, ascends in the mesencephalon and contributes to the innervation of the ipsilateral cortex with thin terminals. The intermediate CA bundle originates from perikarya located in the ventral group of CA pontile neurons (n. subcoeruleus), ascends in the mesencephalic reticular formation, enters the subthalamus and the hypothalamus. Some fibres cross in the supra-optic decussation. This system contributes to the innervation of the hypothalamus, septal region and ipsi and control lateral cortices with thick terminals. The ventral bundle, originating from the medulla, ascends ventrally to the perikarya of the locus coeruleus where it sends some collaterals. It contributes more rostrally to a 'nebula' like formation composed of terminal varicosities in the mesencephalic reticular formation. This formation receives also numerous collaterals from the dorsal and the intermediary bundles. The most rostral part of the ventral bundle has not yet been mapped out. Finally, the DA containing neurons have been mapped out also in the substantia nigra and the nigro striatal system is similar to that described in the rat.

Most of the work which has been carried out in our laboratory has been directed

to the effect of destruction of either DA or NA containing perikarya or of the dorsal and intermediary CA ascending pathways.

NEUROPHARMACOLOGICAL APPROACH TO THE CA NEURONS

The following experiments have been selected in order to demonstrate the complexity of the effects induced by a theoretically simple experiment, i.e. the selective inhibition of CA synthesis.

In normal cats, the inhibition of the biosynthesis of CA, at the level of tyrosine hydroxylase, with alpha-methyl-*p*-tyrosine (AMPT) induces a temporary hypersomnia with increase of both slow wave sleep (SWS) and paradoxical sleep (PS) (KING and JEWETT, 1971). The interpretation of this effect is not easy since it has been shown that the decrease of endogenous cerebral CA which follows the administration of AMPT is accompanied by an acceleration of serotonin (5 HT) turnover (as shown by the increase of 5 HIAA and of 5 HT synthesis) (STEIN *et al.*, 1973). Was the increase of sleep after AMPT due to the inactivation of some CA neurons or to the activation of 5 HT neurons? In order to answer this question, AMPT was given to insomniac cats which raphe system has been previously destroyed (JOUVET, 1969, 1972). In these conditions, the inhibition of CA synthesis was followed by a dramatic temporary decrease of waking (but not by hypersomnia since PS did not appear). Thus, there is some indirect evidence that, even when the 5 HT system is inactivated by lesion, AMPT, by decreasing the turnover of CA neurons (see PUJOL *et al.*, 1973) may suppress temporarily waking. Thus it appears that the hypnogenic effect of AMPT may be mediated by (at least) two synergetic mechanisms: the inhibition of some CA mechanism related to waking and the increase of the turnover of the 5 HT neurons related to SWS and the priming of PS.

EFFECTS OF 6-HYDROXYDOPAMINE

Effect of micro-injection of 6-hydroxydopamine in the dorso-lateral part of the pontine tegmentum

The micro-injection of 6-OHDA in the locus coeruleus and subcoeruleus, with the technique and the dose described by UNGERSTEDT (1969), has given the following results: during the first 4 days there is an increase of the PGO activity (reserpinic syndrome) during waking and SWS, whereas the frequency of PGO during PS decreased together with the daily percentage of PS. After the 6th or 7th day, PS and PGO are totally suppressed. In the cats sacrificed on the 8–10th day, there is a 30–40 per cent decrease of NA in the mesencephalon and telediencephalon but also a similar decrease of 5 HT which indicates that some 5 HT neurons of the raphe system might have taken up 6-OHDA. The long delay (3–4 days) which is necessary for suppressing PS suggests that some neurons of the locus coeruleus or subcoeruleus might be relatively resistant to the effect of 6-OHDA. In control cats in which microinjections of Ringer solution at the same pH are given, PS decreases only by 30 per cent as compared with preinjection controls (BUGUET *et al.*, 1970).

These experiments do not present the crucial proof that only CA-containing neurons of the pontine tegmentum are responsible for PS since the mechanical lesions which follows any microinjection into the brain might have affected other neurons.

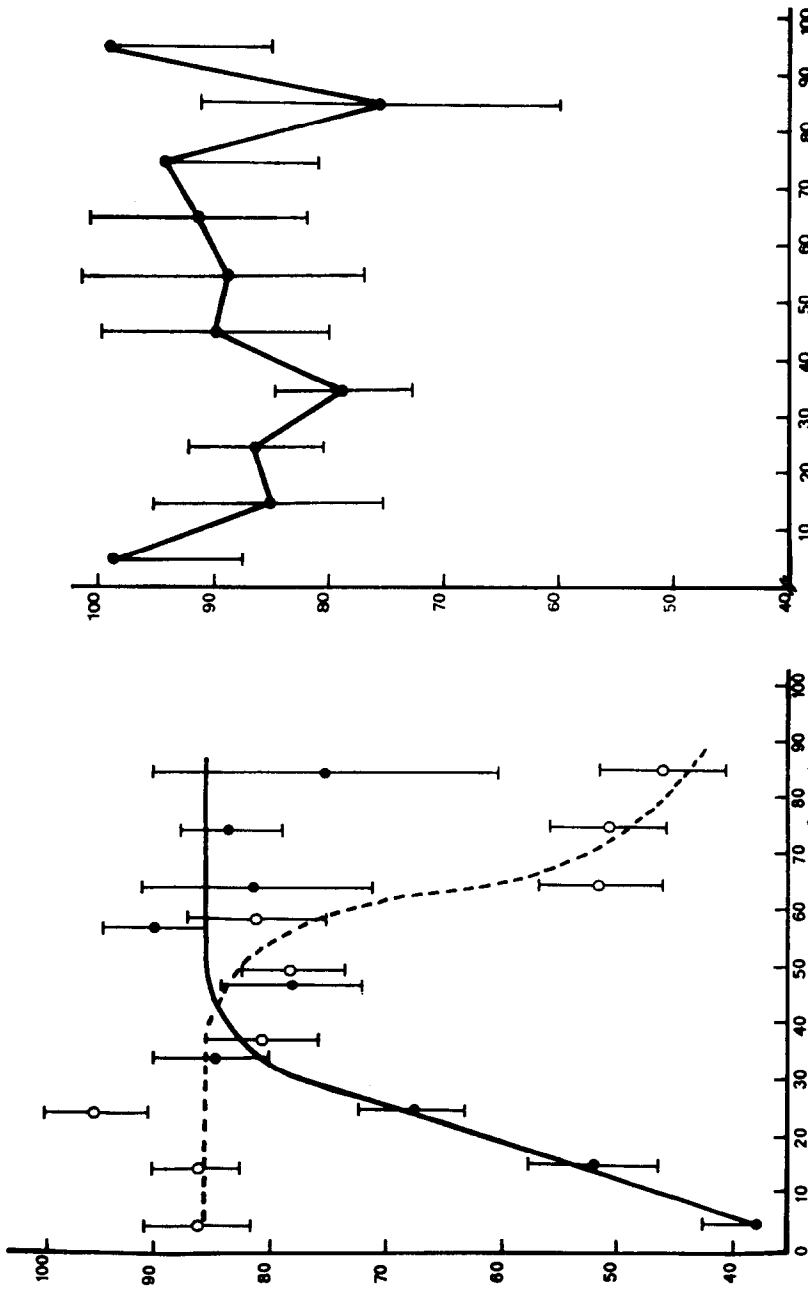

FIG. 1.—On the left hand, ordinate: percentage of 5-HT (black circles) and NA (white circles) in the telediencephalon relative to control cat (mean and S.E.M.. Abscissa: percentage of cortical synchronisation during the 10–13 days of survival after the lesion. The numbers in brackets refer to the number of cats in each group. Other explanations in the text. The absolute value (100 per cent) for 50 control cats are respectively 622 ng/g ± 22 for 5-HT and 399 ng/g ± 10 for NA. On the right, percentage of DA at the level of striatum and telediencephalon. The absolute values for control cats are 947 ng/g ± 41 for DA.

Effect of intraventricular injection of 6-OHDA

Contrary to the rat, 6-OHDA when injected intra-ventricularly in the cat, at a dose of 0·3–2·5 mg induces a decrease of both CA, 5 HT and 5 HIAA. However, the pretreatment of the cats with chlorimipramine protects 5 HT neurons. Thus it is possible to inactivate 'selectively' only CA terminals. In this case, there is a long-lasting (at least two weeks) increase of cortical synchronisation, i.e. decrease of cortical arousal, which is accompanied by a dose-dependent decrease of PS (PETITJEAN *et al.*, 1972, LAGUZZI *et al.*, 1972). This result suggests that some CA mechanisms might be involved in the control of both cortical arousal and paradoxical sleep but it does not reveal what specific system of CA neurons are involved. This problem has been approached by lesioning either the perikarya or the CA ascending pathway.

NEUROPHYSIOLOGICAL AND NEUROCHEMICAL APPROACH

CA perikarya

(i) *The mesencephalic DA-containing perikarya.* The destruction of dopamine-containing neurons of the substantia nigra (group A_9) produces a behavioural state of akinesia and unresponsiveness and a decrease in endogenous DA in the rostral brain (telencephalon, striatum). In some severely lesioned cats, in which DA is severely decreased (by more than 90 per cent) the behaviour is almost permanently comatous. Despite such a depressed behaviour, a quantitatively and qualitatively normal EEG record of alternating paradoxical sleep, slow wave and arousal activity may persist during the behaviourally comatous state (JONES *et al.*, 1968, JONES, 1969).

In view of the numerous anatomical data which suggest the existence of a dopaminergic nigro-striatal ascending system, it appears possible that DA could play a role in behavioural alterness, and motor coordination presumably by way of this nigro-striatal system. On the other hand, the DA-containing neurons do not play a significant role in the regulation of the cortical EEG since an almost total disappearance of dopamine from the tele-diencephalon does not induce a significant change in the spectrum of the EEG recording during the sleep-waking cycle.

(ii) *CA-containing perikarya of the dorso-lateral pontine tegmentum and PS.* The CA-containing neurons of the dorso lateral pontine tegmentum (group A_5, A_6, A_7) are concentrated in the locus coeruleus, subcoeruleus and adjacent nuclei. From a series of more than 100 chronically implanted cats in which coagulations were systematically made in these groups and in control regions, the following pictures of a very intricated system of neurons, leading to descending and ascending CA-containing axons can be summarised (JOUVET and DELORME, 1965, ROUSSEL, 1967, BUGUET *et al.*, 1970) (See references in JOUVET, 1972).

The bilateral lesion of the caudal part of the nucleus locus coeruleus suppresses only the motor inhibition which takes place during PS. This explains why around 8–10 days after the lesion, hallucinatory behaviour may occur periodically during SWS. The ascending components of PS still occur (activated EEG, PGO activity, rapid eye movements, myosis, total relaxation of nictitating membranes). However, the cat standing up looks awake since it may attack unknown enemies, play with an absent mouse, or display a flight behaviour. There exist orienting movements of the head, whereas the animal does not respond to visual or auditory stimuli. These

'pseudo-hallucinatory' episodes (which are good arguments that 'dreaming' may occur during PS in the cat) last for 3–5 min and result in sudden awakening and return to SWS.

After lesion of the caudal part of the locus coeruleus there are no significant alterations of CA endogenous content in the mesencephalon or telediencephalon, but a 30–40 per cent decrease of NA occurs in the cervical spinal cord. Thus, it is possible that some descending CA neurons might be implicated in the control of total decrease of muscle tone which occurs during PS.

More extensive bilateral lesions, involving the caudal half of the locus coeruleus and the N. subcoeruleus suppress definitively the occurrence of PS and 'pseudo-hallucinatory behaviour'. However, some PGO activity may still occur during slow wave sleep. This lesion induces a decrease of about 30 per cent of NA in the telediencephalon without any alteration of the 5 HT or DA levels.

Control lesions located either ventrally to these groups of neurons, or medially, or laterally or caudally (the entire group of vestibular nuclei) do not result in significant alterations of SWS, PS or of the amine content of the rostral brain.

From these data it may be concluded that most of the neurons located in the dorso lateral part of the pons play a role in the execution of PS and that there is a very complicated organisation which is not yet understood. The most caudal part of the pontine CA neurons is related to descending mechanisms, whereas phasic PGO activity and the fast EEG activity appears to depend upon most of the neurons located in the ventral and caudal part of the locus coeruleus and most of the subcoeruleus. Only the group of CA neurons located in the anterior third of the locus coeruleus does not participate in the PS mechanisms, but this group which sends neurons in the dorsal NA bundle is apparently concerned with the control of waking.

CA ascending pathway

(i) *Dorsal NA pathway.* The bilateral destruction of the dorsal NA pathway *at the level of the isthmus* induces a temporary dramatic hypersomnia with an increase of SWS and PS (up to 400 per cent). It is likely that this effect could be due to the suppression of some CA fibres 'controlling' directly or indirectly the anterior part of the raphe system, since there is an increase of 5 HT turnover after this lesion (PETITJEAN and JOUVET, 1970) (see PUJOL *et al.*, 1973).

The lesion of the dorsal NA pathway more rostrally than the isthmus (in the mesencephalon) decreases the duration of cortical arousal without interfering with the phasic short-lasting arousal which usually follows sensory stimulation. This decrease of cortical arousal (or increase of cortical synchronisation) is not accompanied by any increase of PS. There is a significant correlation between the decrease of telencephalic NA which follows the lesion of the dorsal NA pathway and the increase of cortical synchronisation (JONES, 1969). Thus the CA-containing neurons ascending in the dorsal NA bundle play a very subtle role in the regulation of the sleep-waking cycle: some of them are involved in the process of the tonic cortical arousal during waking (hence their inactivation leads to 'unwaking' state), others may control tonically the 5 HT neurons of the rostral raphe system (hence their selective inactivation leads to hypersomnia with increase of both SWS and PS).

(ii) *Lesion of the ascending intermediary CA pathway.* The destruction of the region of the intermediary pathway by electrocoagulation, at the level of the isthmus

suppresses the PGO activity in the lateral geniculate and occipital cortex (LAURENT *et al.*, 1972). This finding is in accordance with the role played by the N. subcoeruleus in the generation of PGO waves. However, the direct proof that only the NA fibres ascending in the region of the intermediary pathway would be responsible for initiating PGO activity has not yet been obtained.

SUMMARY

Three different systems of CA-containing neurons appear to be involved in the regulation of the sleep-waking cycle.

(1) The system originating from the central and caudal part of locus coeruleus and subcoeruleus is concerned with the executive mechanisms of PS, whereas the 'priming mechanisms' of PS (probably serotoninergic) are apparently located in the medial and caudal raphe system (see references in JOUVET, 1972).

(2) The anterior NA pontile waking system originates from the anterior part of the locus coeruleus. This system sends axons to the dorsal NA bundle which ascends near the grey matter. The destruction of the dorsal NA bundle at the level of the isthmus is followed by a significant increase of both states of sleep, whereas the decrease of NA is accompanied by an increase of 5 HT turnover. It is thus possible that some collaterals issued from the anterior nucleus locus coeruleus might exert a control upon the rostral raphe system during waking. The suppression of this control by a lesion would cause an increase in activity of the raphe system leading to a temporary increase of both SWS and PS.

(3) Other neurons ascending in the dorsal NA system in the mesencephalon contribute to the control of cortical arousal. It is not yet known if this effect is directly mediated at the cortical level, or through some connections with the terminal varicosities located in the mesencephalic reticular formation. The destruction of this system decreases cortical arousal but there is no increase of PS. Thus the destruction of the NA neurons in the mesencephalon decreases EEG waking but does not induce a true hypersomnia.

(4) The substantia nigra and the nigro striatal system are apparently concerned with the control of waking behaviour through the extra-pyramidal system, but are not concerned with the regulation of cortical activity during the sleep-waking cycle.

Thus the NA anterior pontile and mesencephalic systems are antagonistic to the 5 HT-containing neurons of the raphe system which is strongly implicated in sleep mechanisms. This is illustrated in the figure which summarises the biochemical data obtained from 133 cats subjected to lesions either of the midline raphe, or of the bulbar, pontine mesencephalic tegmentum or of the substantia nigra. Control lesions were also made outside CA-containing neurons, while 12 sham operated cats served as control (groups of animals are classified according to the percentage of EEG synchronisation during the 10–13 days of the postoperative survival and the mean value of the percentage of 5 HT NA and DA in the telencephalon and diencephalon for each group is given. It is clear that 5 HT and NA fit two opposite curves, while DA does not change significantly. Cats with insomnia have a decreased telediencephalic 5 HT and normal telencephalic NA content. Cats with normal or subnormal levels of synchronisation have normal or subnormal values for both 5 HT and NA, whereas cats with increased synchronisation have a decreased NA with normal or subnormal 5 HT level in the telediencephalon.

These results demonstrate the complexity of the study of the role of CA containing neurons in the brain stem of the cat. More and more converging data from neurophysiology, neuropharmacology and from biochemistry strongly suggest however that the CA containing neurons located in the pons or in the mesencephalon are implicated in the regulation of the sleep-waking cycle in the cat, either directly or indirectly by acting upon serotoninergic mechanisms.

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