Reports

Functional Organization of the Brain Stem Reticular Formation and Sensory Input

Abstract. Injections of 20 μg of adrenaline, dissolved in 10 μl of Tyrode solution, directly into the brain stem reticular formation, are followed by a transient increase in the amplitude of the cortical-evoked response obtained from single-shock stimulation of the optic chiasma, while injections of the same amount of acetylcholine produce a transient decrease. Furthermore, injections of procaine in the medial region of the rostral pontine reticular formation are followed by a long-lasting increase in the amplitude of these evoked responses, while the same injections at mesencephalic levels produce a marked decrease. These findings are interpreted in terms of the presence, in the reticular formation, of two antagonistic ascending systems: one adrenergic, the other cholinergic, whose *tonic* activity originates in the mesencephalic tegmentum in one case and in the caudal regions of the brain stem in the other.

It has been suggested recently that the cellular aggregates of the brain stem reticular formation may contain a "synchronizing" or "sleep-inducing" system of neurons whose function might be antagonistic to that of the activating system (*1*, *2*). In the context of this hypothesis, the level of arousal and electrocortical activity at any given time would be the result, at least in part, of an equilibrium between these two opposite influences acting on diencephalic and telencephalic structures. While the mesencephalic and rostral pontine reticular formation is still considered to be the anatomical substrate for an activating or desynchronizing mechanism, the evidence obtained in cats with complete (*1*) or partial (*2*) sections of the brain stem suggests that the tonic synchronizing influence probably originates from the caudal pontine or bulbar reticular regions. Furthermore, while the activating system is thought to be adrenergic (*3*), it has been proposed, on the basis of results obtained recently in our laboratory, that the "synchronizing" system may use a cholinergic type of transmission, since injections of small quantities of acetylcholine directly into the reticular formation are usually followed by electroencephalographic (EEG) synchronization and sleep (*4*).

It has also been generally accepted, in recent years, that the reticular formation can exert some type of control over specific sensory input, even though the exact nature of this control and its precise sites of action are still matters of controversy. Our working hypothesis was that if a "synchronizing" system did exist in the reticular formation, its effect on sensory input might be opposite to that of the activating system. The cortical-evoked potential obtained from single-shock stimulation of the optic chiasma was chosen as test object, since it has been clearly demonstrated that electrocortical desynchronization after electrical stimulation of the reticular formation will increase its amplitude significantly (*5*).

The experiments were carried out on unanesthetized "encéphale isolé" cats or on cats immobilized with gallamine (*6*). Single-shock, square-wave pulses were delivered to the optic chiasma at a frequency of 1 pulse every 3 seconds, and the evoked potential, recorded from the visual cortex of the posterolateral gyrus, was displayed on a cathode-ray oscillograph. After the photographic recording of 20 or 40 of these evoked potentials to establish base-line levels, small quantities of adrenaline, acetylcholine, or procaine (*6*) were injected slowly, directly into the brain stem reticular formation, and further recordings were made of their effects in the amplitude of the cortical potentials. The drugs were dissolved in 10 μl of a Tyrode solution at a *p*H of 7.4 and each injection was normally preceded or followed by the injection of the same volume of solvent alone for control purposes. Continuous EEG records were obtained on an Offner electroencephalograph throughout the experiment. Injection sites were subsequently controlled on histological sections.

The results obtained are summarized in Fig. 1. When acetylcholine and adrenaline were injected in the same regions of the pontine and bulbar reticular formation, opposite effects were observed. Effective injections of 20 μg of adrenaline were followed by a transient but significant increase in the amplitude of the evoked potentials. These changes in amplitude became apparent towards the end of the injection period, and in all cases within 1 to 2 minutes after its termination, and lasted 2 to 4 minutes before returning to base-line levels (dotted curve, Fig. 1*A*). In contrast, injections of 20 μg of acetylcholine produced a transient decrease, which bore the same general time relationship to the injection as the adrenaline effect but was on the whole longer lasting (solid-line curve, Fig. 1*A*). In one-half of the effective acetylcholine injections, the reduction in amplitude of the evoked potential was preceded by a short-lasting increase which began early during the injection and ended with the customary fall below base-line levels within 1 to 2 minutes after the termination of the injection (not shown in Fig. 1*A*). The exact nature of this momentary and inconstant increase is not known. It corresponds in time to the short-lasting EEG desynchronization which accompanies the salivation, retching, and vomiting observed in the same proportion of cases after acetylcholine injections in intact, freely moving animals (*4*). For this reason, and since the increase in the evoked potential occurs only for the more caudal injection sites and is followed by the usual fall below base-line levels, we believe that it may be the equivalent secondary electrocortical phenomenon in a preparation in which overt manifestations of nausea cannot be observed. It will be possible to verify this supposition in the chronic preparation.

The changes in the amplitude of the evoked response after adrenaline and acetylcholine injections were interpreted as the outcome of the phasic excitation of two antagonistic, rostrally oriented systems. That acetylcholine produces its effect by firing neurons belonging to an inhibitory system, rather than by a direct inhibition of activating neurons, is supported by the observation that procaine injected at the same locus produces the opposite effect, that is, a long-lasting increase in the amplitude of the evoked response (dash-dot curve, Fig. 1*A*). However, this conclusion does not preclude the possibility that the inhibitory system as a whole, as stimulated by acetylcholine, might

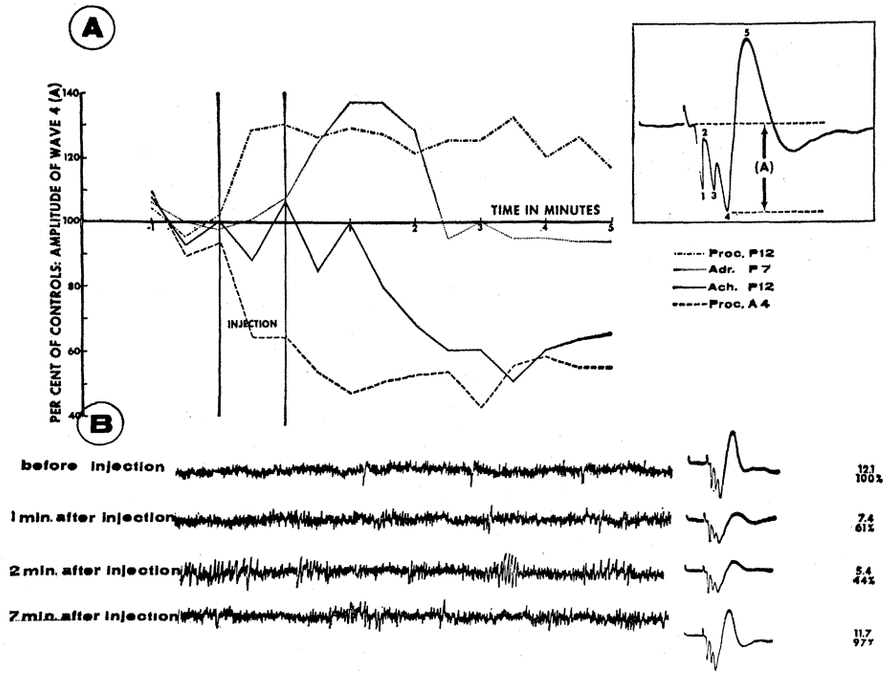

Fig. 1. (*A*) Changes in the amplitude of wave 4 of the cortical responses evoked by single-shock stimulation of the optic chiasma after injections of drugs directly into the brain stem reticular formation. Each point on the curves represents the average of ten evoked potentials expressed as a percentage of the preinjection control level considered as 100 percent. (*B*) Temporal dissociation between the change in the amplitude of the evoked response and the electrocortical changes after an injection of acetylcholine. The figures in the right-hand column represent the average amplitude of the evoked potential during the period of recording shown both in arbitrary and percent values. (For further explanation see text.)

affect thalamic and cortical electrophysiological events only secondarily, that is, by first producing an inhibition of the reticular activating system.

To check this possibility, correlations were established between electrocortical changes and variations in the amplitude of the evoked response after acetylcholine injections. Two main situations emerged. In one, both phenomena showed parallel changes, that is, the EEG became synchronized as the evoked potential decreased in amplitude. This parallelism was typical of the more marked changes in the evoked response. It undoubtedly speaks in favor of the inhibition of the reticular activating system by the cholinergic neurons. In the other situation, a temporal dissociation was observed between the two phenomena, that is, a change in the amplitude of the evoked response occurred in the absence of noticeable electrocortical changes, though slowing of the EEG was usually observed some time later. Such a dissociation is illustrated in Fig. 1*B*. One minute after the injection of acetylcholine the evoked potential has already decreased to 61 percent of its original value without any visible change in the electrocortical trace. It is only 2 minutes after the injection that a slowing of the EEG can be seen, accompanied by a further reduction of the evoked potential. Moreover, 7 minutes after the injection the evoked potential has regained its original amplitude, while the electrocortical activity still shows bursts of slow waves. These observations were taken as an indication that the reticular cells fired by the acetylcholine injection can probably affect thalamic and cortical electrophysiological events directly, and not only through an inhibition of the activating system.

When procaine was injected in the medial region of the rostral pontine reticular formation, the amplitude of the evoked potential was consistently increased and this increase was accompanied by EEG desynchronization. Similar, but less marked and less constant results, were obtained from more caudal injection sites. The changes in the evoked potential usually began early during the injection period and lasted up to 20 minutes at least, this being the longest period of observation (dash-dot curve, Fig. 1*A*). In contrast, injections of novocaine in the rostral mesencephalic tegmentum were always followed by a dramatic fall in the amplitude of the evoked response, accompanied by EEG synchronization or flattening of the electrocortical trace (dashed curve, Fig. 1*A*). The amplitude increase and electrocortical activation obtained from pontine or more caudal injection sites were interpreted as the result of the removal of a tonic inhibitory influence with a consequent release of the reticular activating system. A similar conclusion was reached recently (*7*) after midpontine pretrigeminal surgical transections of the brain stem resulted also in an increase in the amplitude of the visual evoked response. On the other hand, the decrease obtained after rostral mesencephalic injections, as well as the concomitant EEG synchronization, appears to be the consequence of a blocking of the reticular activating system itself.

Negative points were encountered which did not respond either to acetylcholine or to adrenaline. It was at times sufficient to lower the cannula 1 to 2 mm to obtain clear-cut changes in the evoked response when a previous injection had given negative or doubtful results. We believe that this relative difficulty in obtaining easily and consistently reproducible results with this technique is due to a combination of factors such as (i) the mildness of this form of topical chemical stimulation, as opposed to the more usual and more powerful high-frequency electrical stimulation; (ii) the state of the preparation or its "readiness to respond" to this mild stimulation, negative results being more frequent in deeply synchronized brain which responded poorly or not at all to peripheral stimulation; (iii) the apparently limited spread of the injected drugs; and (iv) the functional organization of the target system which may not be homogeneous with respect to the distribution of its cholinergic and adrenergic receptive sites. In spite of its drawbacks, we feel that this method holds promise for future studies because of its theoretically high degree of selectivity, in a nonhomogeneous structure, as opposed to the indiscriminate form of stimulation provided by high-frequency electrical pulses (*8*).

JACQUES COURVILLE
JOHN WALSH
J. PIERRE CORDEAU
Department of Physiology, University of Montreal, Montreal, Canada

References and Notes

1. C. Batini, G. Moruzzi, M. Palestini, G. F. Rossi, A. Zanchetti, *Arch. Ital. Biol.* **97**, 1 (1959).
2. J. P. Cordeau and M. Mancia, *Electroencephalog. Clin. Neurophysiol.* **11**, 551 (1959).
3. M. Bonvallet, P. Dell, G. Hiebel, *ibid.* **6**, 119 (1954); A. B. Rothballer, *ibid.* **8**, 603 (1956).
4. J. P. Cordeau, A. Moreau, A. Beaulnes, *Excerpta Med.* (Intern. Congr. Ser. **37**, 22 (1961); ———, C. Laurin, *Arch. Ital. Biol.*, in press.
5. S. Dumont and P. Dell, *Electroencephalog. Clin. Neurophysiol.* **12**, 769 (1960); F. Bremer and N. Stoupel, *Acta Neurol. Psychiat. Belg.* **58**, 401 (1958).
6. The drugs injected were gallamine triethiodide (Flaxedil), Poulenc Laboratories, Montreal; L-adrenaline bitartrate, K and K Laboratories, New York; acetylcholine bromide, Eastman Organic Chemicals, Rochester, N.Y.; and novocaine, Winthrop Laboratories, Aurora, Ontario.
7. V. Armengol, W. Lifschitz, M. Palestini, *J. Physiol. London* **159**, 451 (1961).

8. Supported by grant MT-841 from the Medical Research Council of Canada.

8 October 1962

Cigarette Smoking and Arteriosclerosis

Recent reports (*1, 2*) have indicated that heavy smokers of cigarettes are more prone than nonsmokers to have heart attacks due to myocardial infarction. The nature of this relationship has not been fully explored, and a number of different interpretations can be suggested.

Myocardial infarcts may be associated with severer symptoms in heavy smokers than in nonsmokers and thus be detected more readily at the time of onset. Although the clinical and electrocardiographic recognition of such lesions has improved greatly, a high percentage of the infarcts found at necropsy are not diagnosed during life (*3*), presumably because they do not cause pronounced or characteristic symptoms. No positive correlation between the incidence of myocardial infarction at necropsy and cigarette smoking has as yet been reported.

A high incidence of myocardial infarction in heavy smokers could depend to some extent on alterations in blood flow due to various mechanisms, and not entirely on the degree of sclerosis of the coronary arteries. In other words, cigarette smokers may be more vulnerable to ischemic infarction than nonsmokers with an equivalent degree of arterial narrowing. Kagan *et. al.* (*4*) suggested that cigarette smoking does not necessarily produce its effect through the pathogenetic mechanism of atherosclerosis but may operate in an indirect fashion to produce symptoms of coronary insufficiency. There is no proof that sclerosis of coronary arteries develops more rapidly in cigarette smokers than in nonsmokers.

The relation between cigarette smoking and heart attacks could be an indirect or even a fortuitous one. Heavy smokers may have other habits or characteristics that make them vulnerable to myocardial infarcts. For example, in an earlier analysis (*5*) it was found that portal cirrhosis of the liver was more common in heavy smokers of cigarettes than in nonsmokers. Further investigation showed that chronic alcoholism was also more prevalent among heavy smokers than among nonsmokers and that the high incidence of liver disease in the former was obviously attributable to this circumstance. It is therefore necessary to determine whether heavy smokers tend to fall into groups having a high incidence of the so-called atherogenic factors, such as diabetes, hypertension, hypercholesteremia, and obesity.

It is possible that cigarette smoking in some selective fashion promotes the development of sclerotic changes in coronary arteries but not in other vessels. No systematic study of the relation of smoking to the severity of sclerotic changes throughout the arterial system appears to have been made. Furthermore, there is little evidence that heavy smoking of cigarettes is associated with a higher incidence of other lesions that may be related to arteriosclerosis, such as scarring of the kidneys, gangrene of the legs, and cerebral infarcts or hemorrhages.

In an earlier report (*6*) a simple comparative method of evaluating the degree of sclerotic change in the aorta at necropsy was described. A record of the smoking habits of most of the individuals of that study was recorded. It was therefore possible to obtain statistical data concerning the relation of smoking practices to the degree of aortic sclerosis. The purpose of the study reported here is to determine (i) whether the clinical evidence of increased incidence of myocardial infarction in heavy smokers is confirmed by a study of necropsy material; (ii) whether there is a higher incidence of other lesions that can be attributed to arteriosclerosis in heavy smokers than in nonsmokers; (iii) whether the degree of generalized arterial sclerosis is a function of smoking habits; and (iv) whether the groups of men with special attributes that make them particularly susceptible to myocardial infarction tend to be heavy smokers of cigarettes.

The study is based on findings in 989 consecutive necropsies on men, performed at the New York Veterans Administration Hospital between 1958 and 1961. In each case the aorta was given an "arteriosclerotic age" through comparison with a set of previously prepared photographic transparencies of aortas which represented the standard or average degree of sclerotic change observed in each half-decade of adult life. If an aorta was evaluated as showing sclerosis characteristic of an age more than 10 years greater than the age of the patient, it was considered to show above-average sclerosis. If it showed sclerosis characteristic of an age more than 10 years less than the age of the patient, it was judged to show below-average sclerosis. Accordingly it was possible to grade each aorta as to whether or not it showed above-average, average, or below-average sclerotic change, regardless of the age of the patient in question. The details of this method of evaluating the degree of atherosclerosis is described more fully elsewhere (*6*).

Daily smoking of more than one and a half packs of cigarettes for many years was the criterion used to define a heavy smoker of cigarettes. Moderate smokers were those who smoked from one to one and a half packs daily. Those who smoked less than a pack a day were listed as light smokers. No attempt was made to separate the pipe and cigar smokers, or to classify them into "light" or "heavy" categories, because this combined group was a relatively small one. Two tobacco chewers are included in this group. When cigarette smokers also smoked pipes or cigars they were classified only according to their cigarette-smoking habits. "Nonsmokers" included a few persons who had smoked briefly early in life. The "unknown or unclassified" group included those for whom no statement about smoking was available and those whose smoking habits had changed drastically over long periods of their lives. The data on smoking were obtained from the routine clinical records. While free from bias, the information available was often lacking in detail. It is possible that some patients who once smoked heavily had ceased to do so after a heart attack in the past and were listed as nonsmokers. In the Framingham-Albany study (*2*), 15 percent of the group were nonsmokers, and in other statistical analyses a comparable percentage were found to be abstainers.