MIDBRAIN INTERACTION WITH THE HYPOTHALAMUS IN EXPRESSION OF AGGRESSIVE BEHAVIOR IN CATS

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Abstract. The effects of injections of M- and N-cholinergic blocking agents into the antero-medial hypothalamus (HM) and the midbrain central gray (GC) on the aggressive behavior of cats, evoked by micro-injections of carbachol into those areas, were investigated in chronic experiments. The influence of pharmacological suppression of the M-cholinergic system in HM on the carbachol-induced aggression response from GC and vice versa was also studied. In the experiments a quantitative method was applied for measuring the specific vocalization — growling, which is a characteristic of aggressive behavior. In the HM and GC areas of the cat the N- and the M-cholinergic systems participated in the control of aggressive behavior, but the M-component dominated in the process. The suppression of M-cholinergic system in GC prevented the appearance of aggressive behavior evoked by injections of carbachol into HM, and the M-cholinergic blockade in HM reduced (by 90%) the aggression response evoked by the injections of carbachol into GC. It is concluded that a concurrent action of the hypothalamic and the midbrain cholinergic systems is necessary for the appearance of a fully expressed aggressive behavior. The hypothalamus and the midbrain are probably links of the same functional circuit, and that the control of aggressive behavior is based on a circulatory action between these structures.

INTRODUCTION

The hypothalamus and the midbrain are, without any doubt, very important links in the central system controlling aggressive behavior (3, 9, 11, 12, 14, 15, 25, 29). Their mutual relations, however, as well the
degree of their participation in the control of such behavior have not been elucidated. As found by Hunsperger (14), the attack response evoked by electrostimulation of the midbrain was not changed after electrolytical destruction of the "hypothalamic defensive zone". On the contrary, lesions of the midbrain central gray matter prevented the appearance of majority of symptoms evoked by electrostimulation of the hypothalamus or even blocked them completely (3, 11, 25, 26). Sheard and Flynn (24) showed that the midbrain stimulation, which caused a slight arousal response when acting alone, facilitated the hypothalamic attack response when both these structures were stimulated simultaneously. Some interesting data were presented by Ellison and Flynn (12): they found that the aggressive behavior of cats was not affected by surgical isolation of the hypothalamus from the rest of the brain. In such preparations it was still possible to obtain a well expressed attack response with all accompanying vegetative and somatic symptoms by electrical stimulation of the midbrain and/or by an external stimulation (body or tail pinching). However, spontaneous defensive responses were never observed in these animals; they were apathetic and hypoactive all the time after the operation. The results failed to confirm the generally accepted idea of the hypothalamus as the main brain center integrating and triggering defensive responses. They suggest that the hypothalamus is not necessary for the initiation and development of aggressive behavior. In further investigations of this preparation Gellen et al. (13) confirmed partially the observations of Ellison and Flynn (12): their cats, though inactive and showing no signs of spontaneous aggressiveness, were able to respond aggressively to external sensory stimulation. This response, however, did not appear after intravenous injections of oxotremorine. On the basis of many investigations it is known that oxotremorine induces a typical aggressive behavior in animals with the hypothalamus intact (1, 13, 17, 28). Furthermore, Berntson (3, 4) has recently shown the existence of separated regions in the midbrain responsible for the appearance of such responses as growling, hissing and ear flattening which are integral parts of aggressive behavior. These responses, typically evoked by electrostimulation of the hypothalamus, did not appear after selective lesions of the midbrain. The results speak in favor of the former opinions (2, 21) suggesting that the hypothalamus evokes aggressive behavior through activation of midbrain and lower brain stem efferent systems responsible for separate fragments of the response (e.g., growling, hissing, piloerection) forming an integrative model of aggression.

As previously mentioned (7, 16) the carbachol-induced aggression response is of a complex nature and proceeds in two phases: phase I — the phase of vocalization (growling and hissing) — rapidly increasing
and short-lasting (about 30–50 min) and phase II — the phase of autonomic changes (the increase of heart rate, blood pressure and respiration) — slowly increasing and long-lasting (60–80 min). It has been observed in this connection that the characteristic vocalization (growling and hissing) is the main and the most specific indicator of the rage state: the cats do not seem to be aggressive when it disappears. All autonomic symptoms (the increase of heart rate, blood pressure and respiration) are less specific and cannot be regarded as an indicator of aggression response, though it is always accompanied by them. Therefore in this paper the analysis of aggressive behavior was based strictly on the vocalization response.

METHODS

The experiments were performed on 14 cats of both sexes of 3.0 to 3.5 kg body weight. Chronic cannulas were implanted bilaterally into the midbrain central gray matter (GC) and the antero-medial hypothalamus (HM) in all cats. The implantation of cannulas was performed in semisterile conditions under barbiturate anesthesia (hexobarbital, 90 mg/kg, intraperitoneally). The cannulas were introduced into the brain with the aid of stereotaxic instrument and the stereotaxic atlas of the cats brain by Snider and Niemer (27). A selfpolymerizing resin (Duracryl, Spofa, Prague) was used for the fixation of the cannulas to the surface of the skull. The outer openings of the cannulas were plugged with cotton swabs soaked with 0.9% of NaCl solution and sealed with wax. The chronic cannulas (1.0 mm outer and 0.6 mm inner diameter) served as guides for an injection cannula (0.5 mm outer and 0.2 mm inner diameter) which was directly connected with a microinjection syringe (E. Zimmermann, Leipzig). Experiments began 10 days after operation. Bilateral intrahypothalamic and intramidbrain injections of different synergistic and agonistic cholinergic agents (isotonic solutions in volume of 1.0 μl ± 0.1 μl into each hemisphere) were made. All substances (obtained in crystaline forms) were dissolved in 0.9% NaCl with the addition of NaH₂PO₄-Na₂HPO₄ buffer to maintain the pH — 7.0 to 7.3.

The following substances were used: carbachol (choline chloride carbamate, Koch–Light), atropine (atropine sulfate, BDH, London) and hexamethonium (hexamethylene-bis-trimethylammonium-chloride, Fluka–Buchs).

The experimental procedure was as follows. After a microinjection the cats were placed in a wooden cage (60 × 50 × 50 cm) with a front wall made of plexiglass to allow the observation of the animals' behavior. All vegetative, somatic and behavioral symptoms appearing after injec-
tions were recorded in detail, more interesting fragments were photographed or filmed. To make the observations of aggressive behavior more objective the number and duration of the very specific vocalization — growls and hissing — were counted. This vocalization, apart from other symptoms, always accompanies the aggressive reaction in the cat and forms its most specific, stable and repeatable indicator (7, 10, 16). A set of telephone digital counters operated manually allowing simultaneous measurements of the number of hisings and growlings as well their duration, was used for the experiments. The pressing of the on-switch of the apparatus (made immediately of the beginning of vocalization) started two different counters. One of them recorded the duration of vocalization in seconds \((\pm 0.1 \text{ s})\) and the other the number of vocalization. Every minute the instrument readings were put on record. The measurements of growling were made for 30 min during each experiment. The number and duration of hisings was not counted because that response was less regular and, moreover, was strongly dependent on different environmental factors (e.g., experimenter's movements). Apart from the measurements made with the use of the counters the whole vocalization (growling and hissing) was recorded on a magnetic tape.

Microinjections were made 3–5 days apart in the following succession:

1. Carbachol into HM, 0.0251 \(\mu\)M solution into each hemisphere;
2. Carbachol into GC, 0.0251 \(\mu\)M solution into each side;
3. Carbachol into HM (2 \(\times\) 0.0251 \(\mu\)M) preceded by the blockade of nicotinic receptors in this area by hexamethonium (2 \(\times\) 0.0366 \(\mu\)M);
4. Carbachol into HM (2 \(\times\) 0.0251 \(\mu\)M) preceded by the blockade of muscarinic receptors in this area by atropine (2 \(\times\) 0.0346 \(\mu\)M);
5. Carbachol into GC (2 \(\times\) 0.0251 \(\mu\)M) preceded by the blockade of nicotinic receptors in this area by hexamethonium (2 \(\times\) 0.0366 \(\mu\)M);
6. Carbachol into GC (2 \(\times\) 0.0251 \(\mu\)M) preceded by the blockade of muscarinic receptors in this area by atropine (2 \(\times\) 0.0346 \(\mu\)M);
7. Carbachol into HM (2 \(\times\) 0.0251 \(\mu\)M) preceded by the blockade of muscarinic receptors in GC by atropine (2 \(\times\) 0.0346 \(\mu\)M);
8. Carbachol into GC (2 \(\times\) 0.0251 \(\mu\)M) preceded by the blockade of muscarinic receptors in HM by atropine (2 \(\times\) 0.0346 \(\mu\)M);
9. Carbachol into HM (2 \(\times\) 0.0251 \(\mu\)M);
10. Carbachol into GC (2 \(\times\) 0.0251 \(\mu\)M).

The procedure was well tolerated and the responses were repeatable. It justified the use of the same cats for experiments during several months.

After completion of the experiments all animals were killed by an
Fig. 1. Photomicrographs of frontal sections of the hypothalamus and midbrain showing the cannula tract endings from which the carbachol-induced response was obtained. The white strip located on the bottom of each photograph consists a scale of 5 mm length.
overdose of anesthetics. Series of 50 μm thick frozen frontal sections of the brains were cut with the use of a microtome and then photographed. The photomicrographs served for verification of the anatomical localization of the injection sites.

Student's t-test was used for statistical evaluation of the results obtained in the course of the experiments.

RESULTS

Histological analysis. Figure 1 presents the photomicrographs of frontal brain sections showing the midbrain and the hypothalamic areas of 14 cats from which the carbachol-induced aggressive behavior was obtained. These sections were selected so as to show the medial part of the areas into which injections were made. As can be seen, the localization of the cannula tips was not the same in all instances: in the case of the hypothalamus they were located between 10.0 and 12.5 frontal planes, and between APO and 2.0 in the case of the midbrain. We have not found in the course of experiments any differences in the cats general behavior that might be correlated to the differences in the location of the cannulas.

The effects of intrahypothalamic and intramidbrain injections of carbachol. Bilateral microinjections of carbachol (0.0251 μM solution into each hemisphere) into HM and GC were made in all cats. Formerly placid and friendly cats after the injection started to growl regularly and hiss sporadically, and their pupils became dilated. Each attempt of provocation (e.g., putting a stick into the cage) was followed by a swift and precise attack directed against the moving object. The growling response obtained after injections into GC had a shorter latency (1 min average, the mean calculated from three experiments performed on 14 cats) than that obtained after HM injections (2 min average, the mean calculated from three experiments performed on 14 cats). In both cases it increased rapidly in magnitude and attained its maximal intensity in 3–5 min after injection. After that time the level of growling did not change during 15–20 min, and then decreased gradually (Fig. 2 and 3). After the discontinuation of growling (30–50 min after injection) cats did not attack moving objects when provoked, though the state of general alertness still persisted. The quantitative analysis of the carbachol-induced growling response (based on the means calculated from the summarized numbers of growls and the summarized time of their duration in the whole group of 14 cats) has shown that the estimated level of this response observed after carbachol injections into GC is higher than that from HM (Fig. 2
and 3). The calculated average (three experiments, 14 cats) are: the number of growls from GC = 182.4 ± 77.8, and from HM = 126.6 ± 58.7, the duration of growling from GC = 408.7 ± 158.1 min, and from HM = 272.2 ± 84.3 min. The calculated standard deviations are comparatively high — the fact was caused by big individual differences between cats. Some of them showed always a “high” and some always a “low” level of vocalization. It should be stressed that the “high” or “low” level of vocalization was a characteristic feature of each animal and was comparatively steady after each injection of carbachol. It is very likely that differences in the location of the cannulas (which obviously could not

Fig. 2. Effect of intrahypothalamic injections of carbachol (1), hexamethonium + carbachol (2), and atropine + carbachol (3) on the growling reaction in cats. Each point represents the mean calculated from the summarized numbers of growls and the summarized time of their duration in the group of 14 cats. Asterisks denote significantly different results (P < 0.01) as compared to control.
be avoided in this type of experiment) were the cause of individual differences in the level of vocalization. The outcome of all calculations obtained in the first part of the experiment was regarded as a 100% and served as a reference point for the data obtained in the next part.

Fig. 3. Effect of intramidbrain injections of carbachol (1), hexamethonium + carbachol (2), and atropine + carbachol (3) on the growling reaction in the cats. Each point represents the mean calculated from the summarized numbers of growls and the summarized time of their duration in the group of 14 cats. Asterisks denote significantly different results \( P < 0.01 \) as compared to control.

The effect of intrahypothalamic injection of cholinergic blocking agents, followed by carbachol injections into the same area. In all cats bilateral injections of hexamethonium (0.0366 μM solution into each he-
misphere) into the HM region were made in order to block the nicotinic receptors, and after 10 min carbachol was injected into the same places (0.0251 μM solution into each hemisphere). We did not notice any changes in animal behavior after hexamethonium injections alone, but after the successive injection of carbachol into the same area a full set of symptoms typically accompanying the aggressive behavior appeared: pupil dilatation, growling, hissing, provoked attack. However, the latency of the

Fig. 4. Influence of muscarinic and nicotinic blocking agents on the carbachol-induced growling response. Number (white columns) and duration (black columns) of growling in per cent of the control. Each column represents the mean calculated from the summarized numbers of growls and the summarized time of their duration in the group of 14 cats. 1, carbachol (0.0502 μM solution); 2, atropine (0.0692 μM solution) + carbachol (0.0502 μM solution); 3. hexamethonium (0.0732 μM solution) + carbachol (0.0502 μM solution); 4. 0.0692 μM solution of atropine injected into the midbrain + 0.0502 μM solution of carbachol injected into the hypothalamus; 4',0.0692 μM solution of atropine injected into the hypothalamus + 0.0502 μM solution of carbachol injected into the midbrain. Asterisks denote significantly different results (P < 0.001) as compared to control.

growling response was longer (about 3 min), the number of growlings decreased to 19.0% and their duration was lowered to 21.3% (P < 0.001) in comparison with the control experiments (Fig. 2 and 4). It became obvious then, that the N-cholinergic blockade caused a serious reduction of the carbachol-induced aggression response. A couple of days later atropine (0.0346 μM solution into each hemisphere) was injected bilaterally into HM of the same cats. After 10 min it was followed by the in-
jection of carbachol (0.0251 μM solution into each hemisphere). No changes in animal behavior were observed either within the 10 min period when atropine acted alone, or after the injection of carbachol. The cats were placid and behaved identically as in the period when no injections were made. Thus, it turned out that the blockade of M-cholinergic receptors in HM prevented the appearance of symptoms evoked by carbachol injection into the same area. It can be concluded on the basis of that result that carbachol injected into HM evokes aggressive behavior through the activation of N-as well M-cholinergic receptors, but the participation of the M-component is more important.

The effect of intramidbrain injection of cholinergic blocking agents, followed by carbachol injections into the same area. In the further part of the experiments hexamethonium was injected into GC area (0.0366 μM solution into each part) in all cats. Ten minutes later it was followed by an injection of carbachol into the same sites (0.0251 μM solution into each part). Similarly to the preceding part of the experiment, no changes occurred within 10 min after injection of hexamethonium, but after a subsequent injection of carbachol, aggressive behavior appeared. The latency of the growling response did not change (1 min), the number of growlings decreased to 64.3%, and the duration of growling lowered to 65.5% in comparison to the control (Fig. 3 and 4). It could be concluded that the blockade of N-cholinergic receptors in GC caused a diminution of the carbachol-induced aggressive response, though to a lesser degree than in the case of HM. After several days atropine was injected into GC (0.0346 μM solution into each part) and followed 10 min later by an injection of carbachol into the same sites (0.0251 μM solution into each part). The cat's behavior did not change after atropine injections, but after the injections of carbachol weak symptoms of aggressive behavior appeared. The number of growlings was 7.6% and the duration of growling 7.8% of the appropriate control values (Fig. 3 and 4). The cats never attacked when provoked. It turned out that the blockade of M-cholinergic receptors inhibited almost entirely the action of carbachol in this area. It might be assumed that, similarly as before, the N- as well M-cholinergic component is involved in the carbachol-induced aggressive behavior from GC, but the M-component is prevailing.

The effect of injections of carbachol into the hypothalamus preceded by a blockade of muscarinic receptors in the midbrain and vice versa. The purpose of that part of the experiment was to study the mutual, functional relationships between HM and GC in control of aggressive behavior. The investigations were performed with the use of the method of pharmacological suppression of one structure with simultaneous che-
mostimulation of the other one. Bilateral injections of atropine (0.0346 μM solution into each part) were made into GC area in all cats and followed 10 min later by injections of carbachol (0.0251 μM into each hemisphere) into HM. No symptoms characteristic for the carbachol-induced aggressive behavior appeared, the growling and hissing were absent, the cats were quiet and did not show any signs of aggression when provoked. It proved clearly that the atropine-induced blockade of M-cholinergic system in GC made the appearance of the carbachol-induced aggression from HM entirely impossible. A couple of days later atropine (0.0346 μM solution into each hemisphere) was injected into HM of the same cats and followed 10 min later by injections of carbachol into GC area (0.0251 μM solution into each part). The atropine injection did not evoke any change in behavior, however, a subsequent injection of carbachol evoked a weak growling response: its duration was 10.0% and the number of growlings was 8.2% of the control value. The results of this experiment proved that the blockade of the M-cholinergic system in HM forms a serious obstacle for the development of the carbachol-induced aggression response from GC, but does not inhibit it completely.

**DISCUSSION**

In the experiments described above we tried to elucidate the functional relationships between GC and HM in the control of aggressive behavior in cats mediated through the cholinergic system. We wanted to find out whether these regions were the links of the same functional circuit, or whether they formed a hierarchical system. It seemed at first that this problem had been resolved univocally by Ellison and Flynn (12) — they had found that the aggression response could be evoked by electrostimulation of the midbrain after the isolation of the hypothalamus from the rest of the brain. This result suggested clearly a crucial role of the midbrain in the control of aggressive behavior. Further investigation, however, questioned the hierarchical organization of the midbrain–hypothalamic system. It was shown by Gellén et al. (13) that intravenous injections of oxotremorine to cats with isolated hypothalamus do not evoke aggressive behavior. Of course, after surgical isolation of the hypothalamus the blood–brain barrier is being broken and peripherally injected oxotremorine can act, without any obstacles, centrally. The results of these experiments have shown that the integrity of all links of the cholinergic system in different parts of the brain is necessary for the development of aggressive behavior. It should be emphasized that a cholinergic character of the triggering mechanism starting the aggression behavior is commonly accepted on the basis of neurochemical in-
vestigations (5, 10, 18, 22, 23, 29, 30). In this situation it seems that the use of pharmacological suppression of neural structures which can be regarded as a kind of selective and reversible lesion, constitutes a good method, particularly convenient for the investigations of functional relationships between different brain regions. It makes possible the repetition of the same experimental operations many times assuring a better accuracy of results. In order to make the evaluation of results more objective, apart from behavioral observations, an exact quantitative measure of the very characteristic vocalization which is a specific component of aggressive behavior (growling) was applied. It served as the main index of the intensity of emotional response (7, 10, 16).

In the course of the experiments we found that the atropine-induced blockade of M-cholinergic receptors in GC prevented completely the appearance of the aggression response induced by intrahypothalamic injections of carbachol. On the other hand, the blockade of the M-cholinergic receptors in HM caused a strong decrease of the carbachol-induced aggression behavior from GC area. The fact that the atropine-induced blockade in GC makes the induction of aggression by an injection of carbachol into HM impossible could support the assumption put forward by Clemente and Chase (9). It suggests that a preliminary integration of sensory stimuli coming from lower parts of the central nervous system occurs in the midbrain. From here they are transmitted to the hypothalamus where the final stage of integration takes place and the hypothalamic rage mechanism is triggered off. This is followed by an activation of the effector elements in the hypothalamus and in the midbrain that are responsible for the appearance of vegetative, somatic and behavioral manifestations of aggressive behavior. On the other hand, a partial suppression of the carbachol-induced aggression response from GC after the atropine-induced blockade in HM could support the hypothesis put forward by Ellison and Flynn (12), who considered the midbrain as an important center controlling the aggressive behavior, unless the atropine injections into GC only partially prevented the appearance of an aggressive response. In our opinion, this result is very interesting though difficult to interpret, and deserves more attention. Possibly, the carbachol introduced into GC, apart of its specific cholinergic action, exerts an unspecific influence (e.g., causing an ionic imbalance) on other neurotransmitter systems and in that way activates different effector systems involved in the expression of aggressive behavior. Such differentiated systems for the growling and hissing responses in the midbrain areas adjoining the aqueduct have been found and described in detail by Berntson (3). He has shown that these different types of vocal responses evoked by electrostimulation of the hypothalamus could be eliminated by
selective lesions of the midbrain. It is very likely that in the experiments performed by Ellison and Flynn (12) on cats with an isolated hypothalamus only some effector systems responsible for the appearance of the symptoms accompanying the aggressive behavior were activated during electrostimulation of the midbrain. The fact that the cats with the hypothalamus isolated were completely inactive and never showed any signs of spontaneous aggression, and, what is most important, that the aggression response evoked by electrostimulation of the midbrain did not have the emotional character which is always present in cats with the hypothalamus intact, might strongly support this supposition. Our results, however, strictly measurable and quantitatively comparable, suggesting that the carbachol-induced aggressive response from GC after the atropinic blockade in HM may be drastically diminished (Fig. 4) and showing that the cats do not attack after a provocation, do not allow to accept the Ellison and Flynn's hypothesis regarding the midbrain as the main center responsible for the control of aggressive behavior (12). On the contrary, our results suggest that a cooperative action of the cholinergic system in the midbrain and the hypothalamus is necessary for the development of the fully-expressed aggressive behavior. It might be then presumed that the midbrain and the hypothalamus form links of the same functional circuit and that the control of aggressive behavior is accomplished on the basis of a circulatory cooperation, but not hierarchical relations.

As it was mentioned above, there is a general agreement (with the exception of the Reis' study (20)) that the cholinergic system is the main system participating in the control of aggressive behavior. However, there are some discrepancies as to which part of this system is the most important. Some authors (5, 6, 18, 19, 29) suggest that in the hypothalamus as well as in the midbrain the aggression response is mediated through the M-cholinergic system. A different viewpoint is represented by Burov and Kurochkin (8): in their opinion in the hypothalamus aggression response is mediated through the M-cholinergic system, but in the midbrain by the N-cholinergic system. Várszegi and Decsi (30) and Decsi et al. (10) presume that the carbachol-induced response is mediated through M- as well N-cholinergic system with the N-cholinergic component being responsible for autonomic symptoms. These discrepancies are probably caused by the use of different blocking agents and, which is more important, by the use of different and unprecise criteria of estimation of aggressive response. In our previous paper (23) we concluded, on the basis of a symptomatic evaluation of the animal behavior after intrahypothalamic injections of carbachol, that in this area aggressive response is mediated by the M-cholinergic system — the blockade of ni-
cotinic receptors with the use of betamone did not prevent the development of different symptoms accompanying the aggressive behavior (pupil dilatation, piloerection, snorting). The results of the present investigations, based not only on a symptomatic evaluation but mainly on an exact measure of the specific vocalization, have shown clearly (experiments II and III) that the carbachol-induced aggression response is greatly diminished after the blockade of nicotinic receptors in the hypothalamus as well as in the midbrain. The greater suppression which was observed after the blockade in the HM area (Fig. 2–4) might suggest that the hypothalamus plays a guiding role in the control of different autonomic activities. The present results allow to conclude that the control of aggressive behavior in the hypothalamus and in the midbrain is accomplished by the M- as well N-cholinergic system, but the muscarinic component is more important.

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