Inhibition of Jaw-Opening Reflex by Stimulation of the Central Amygdaloid Nucleus in the Cat

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Summary The effect of stimulation of the amygdaloid complex on the jaw-opening reflex (JOR) was studied in the cat anesthetized with pentobarbital sodium. The conditioning stimulation of the central amygdaloid nucleus (ACE), but not the other amygdaloid nuclei, markedly inhibited the JOR induced by the tooth pulp stimulation. Ipsilateral ACE conditioning stimulation with 300 μ A produced inhibition which lasted approximately 500 ms from the cessation of the stimulation. Additionally, the microinjection of monosodium glutamate into ACE elicited inhibition of the JOR that lasted about 10 min. These findings suggest that the excitation of the cell bodies in ACE exerts inhibitory action on the trigeminal nociceptive reflex.

Key words: central amygdaloid nucleus, tooth pulp, jaw-opening reflex.

The amygdaloid complex contains high levels of opiate receptors and enkephalins which are involved in antinociception in the central nervous system (KUJAR *et al.*, 1973) and has direct afferent connections via the ventral amygdalofugal fibers with the periaqueductal gray (PAG), nucleus raphe magnus (RAM), and locus coeruleus (HOPKINS and HOLSTEGE, 1978; PRICE and AMARAL, 1981) which are concerned in the descending control of nociceptive transmission (for a review, FIELD and BESSON, 1988). Furthermore, the microinjection of neurotensin or morphine into the central amygdaloid nucleus (ACE) produced a significant increase in the nociceptive threshold (RODGERS, 1978; KALIVAS *et al.*, 1982). We assumed, therefore, that the amygdala may play a role in the modulation of nociception. As the jaw-opening reflex (JOR) in animals produced by electrical stimulation of the tooth pulp has been used as a reflex measure of pain responsibility (MASON *et al.*, 1985), this study sought to determine if the conditioning stimulation of the amygdaloid complex, particularly the central nucleus

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(ACE), exerts an influence on the digastric EMG activities (JOR) induced by tooth pulp stimulation. That is, is the amygdaloid complex involved in the trigeminal nociceptive reflex?

Nineteen adult cats weighing 2.5–4.3 kg were used. The animals were initially anesthetized with ketamine (25 mg/kg, i.m.) for surgical preparation and treated with atropine sulfate (0.2 mg/kg, i.p.). The anesthesia was maintained by a periodic intravenous dose of pentobarbital sodium (5–10 mg/h). Small cavities were drilled into the dentine of the upper and lower molars bilaterally and a stimulating electrode (small screw) with a lead wire was implanted into each cavity. The head of the screw and the surrounding area were covered with polycarboxylate cement and acrylic resin. A single rectangular pulse, 0.5 ms in duration, was bipolarly delivered to the tooth pulp and the intensity was maintained at 1.2–1.5 times the threshold for the JOR.

A concentric bipolar electrode for the conditioning stimulation was placed stereotaxically in the amygdaloid complex (*A*: 11.0–13.0; *L*: 8.0–12.0; *H*: –1 to –15). The stimulation was a train of 33 rectangular pulses (0.5 ms duration) at a frequency of 330 Hz at intervals of 8–10 s with an intensity of 50–400 μ A. The stimulating side of the amygdaloid complex was usually ipsilateral to the EMG recording side.

The microinjection of monosodium glutamate (500 mM in distilled water) into the ipsilateral ACE was conducted via needle of microsyringe (25 μ l) attached to the stimulating electrode. The glutamate was injected exactly 500 μ m above the most effective sites for inhibition by electrical conditioning stimulation of ACE. Glutamate (10 μ l) was injected slowly over 1 min by manual pressure.

EMG activities from the digastric muscle were recorded bipolarly by a needle electrode and fed into an amplifier. The signals were then averaged ten times by a computer (Nihon Kohden, QC-111J). The stimulating sites in the amygdaloid complex were marked by depositing iron from the electrodes with an anodal current. At the termination of these experiments, animals were sacrificed with an overdose of pentobarbital sodium and then perfused with saline followed by 10% formalin containing 2% potassium ferrocyanide. Frozen coronal sections of 50 μ m thickness were taken, and then stained with cresyl violet. The stimulating sites were determined by histological examination of the serial sections.

The intensity of the tooth pulp stimulation which was equal to 1.2–1.5 times the JOR threshold had a mean ($\pm$ S.D.) latency of 7.79 ± 0.56 ms ($n=7$). There was almost no difference between the latencies for the maxillary and mandibular tooth pulps.

Since conditioning stimulation of the lateral amygdaloid nucleus and peri-amygdaloid area sometimes evoked the jaw-closing movements, JOR was inhibited by the stimulation. These data were excluded from the analysis. ACE stimulation alone did not evoke the EMG response in the digastric muscle, but the conditioning stimulation of ACE markedly reduced the amplitude of the digastric EMG response elicited by tooth pulp stimulation (Fig. 1). As compared with the side contralateral

Fig. 1. Location of the conditioning stimulation in the ipsilateral central amygdaloid nucleus (ACE) and the conditioning effect on JOR. The photomicrograph indicates that the stimulating site (arrowhead) is located in the medial division of ACE. The lower part of this figure shows the inhibitory effect of the ACE conditioning stimulation ($300 \mu\text{A}$) on the digastric EMG response to tooth pulp stimulation (lower molar, $70 \mu\text{A}$). The JOR response was inhibited at a conditioning-test (C-T) interval of 110 ms. ABL, basolateral amygdaloid nucleus; ABM, basomedial amygdaloid nucleus; ALA, lateral amygdaloid nucleus; CL, claustrum; GP, globus pallidus; OT, optic tract; PAM, periamygdaloid area; PU, putamen. Calibration: $40 \mu\text{V}$, 10 ms.

Fig. 2. Effects of conditioning stimulation of the ipsilateral ACE on JOR elicited by tooth pulp stimulation at various C-T intervals ($n=7$). All points in the diagram indicate mean percent change in amplitude of EMG response with conditioning stimulation vs. control. The vertical bars at each point indicate the standard deviation. A significant inhibition was obtained even at C-T interval of 1,000 ms (Wilcoxon test, $p<0.05$). The abscissa shows the C-T interval in ms.

to the EMG recording side, the ipsilateral ACE stimulation with the same intensity more markedly inhibited JOR. Likewise the medial division of ACE showed predominantly more inhibition than the lateral one. The mean current to produce a 50% inhibition of the control amplitude was $87.5 \mu\text{A}$ for the ipsilateral conditioning stimulation and the stimulation with $300 \mu\text{A}$ elicited the maximal amount of inhibition ($n=5$). Therefore, the intensity of $300 \mu\text{A}$ was used in the ACE conditioning stimulation in the following experiments. Figure 1 represents a typical example in which the magnitude of the amplitude reduction was related to the interval between the conditioning and test stimuli (C-T interval). At a C-T interval of 110 ms the response was almost completely extinguished by the ACE conditioning stimulation, but recovered to the control level at a C-T interval of 500 ms.

Figure 2 indicates the time course of the inhibitory effect of ACE conditioning stimulation ($300 \mu\text{A}$) on JOR in 7 trials in 7 animals. The percent of the control JOR to tooth pulp stimulation is plotted against the C-T interval. This inhibitory effect reached its peak (9.2% of control) at 10–20 ms from the cessation of the conditioning stimulation (C-T interval of 110–120 ms) and then gradually recovered to 89.7% of the control value at a C-T interval of 700 ms.

Monosodium glutamate ($10 \mu\text{l}$) (500 mM) was injected into ACE in 4 experiments (Fig. 3). This was done to determine whether the inhibition of JOR induced by the ACE electrical stimulation was caused by the excitation of the passing fibers or the cell bodies in ACE. The JOR was suppressed by the glutamate injection. Figure 3 shows that the inhibitory effect reached its peak (48%) within 80 s post injection and the JOR recovered to the control level at about 9 min. In the other 3 experiments, the peak of the inhibitory effect was also within 6 min and the effect

Fig. 3. Effects of glutamate microinjection ($10\mu\text{l}$) into the ipsilateral ACE on the JOR elicited by tooth pulp stimulation. In ACE (large arrowhead), both the electrical stimulation and glutamate injection induced an inhibitory effect. In the globus pallidus (small arrowhead), the electrical stimulation induced a remarkable inhibitory effect, but glutamate injection did not elicit any effect. The first deflection in each potential is the stimulus artifact. IC, internal capsule; EN, entopeduncular nucleus; the other abbreviations in the upper figure are the same as in Fig. 1. Calibration: $50\mu\text{V}$, 10 ms.

lasted about 10 min. The injection of glutamate (same dose) into tissue adjacent to the ACE, including the lateral, basolateral amygdaloid nuclei, and globus pallidus did not induce the inhibition of JOR. Likewise, the injection of 10 μ l distilled water into ACE did not exert influence on the JOR.

It is likely that the effective current spread was not more than 500 μ m, because the stimulation of sites 500 μ m distant from effective sites in ACE in the same animals was not effective. The glutamate injection caused an inhibition of JOR similar to that caused by electrical stimulation of the ACE. These findings suggest that the inhibition of JOR by electrical stimulation was elicited by the excitation of the cell bodies rather than of the passing fibers in the limited area of ACE. The present finding that stimulation of medial division was more effective than that of the lateral division agrees with the anatomical observation that only the medial division projects to the brainstem (HOPKINS and HOLSTEGE, 1978).

Contrary to our results, it was reported in the rat that ACE stimulation induced activation of a majority of mylohyoid-anterior digastric motoneurons and inhibition of approximately one-third of the masseteric motoneurons (SASAMOTO and OHTA, 1982; OHTA, 1984). Differences in the anesthetic, the species of animals, or the sites stimulated might account for the discrepancy between their observations and ours. And it is very possible that the effects depend on the excitation of the fibers in ACE and/or the neighboring area, because they employed a high intensity electrical stimulation and did not use the excitants to excite the cell bodies. Their results are in agreement with Gary Bobo and Bonvallet's findings (GARY BOBO and BONVALLET, 1975) in the cat, in which the stimulation of ACE induced both facilitatory and inhibitory effects on the masseteric reflex; the facilitatory effects resulted from both fascicularis longitudinalis associations and ansa lenticularis, and the inhibitory effects resulted from the ansa lenticularis. There is another possibility that ACE stimulation may facilitate the JOR at C-T interval shorter than 110 ms, because we did not examine the conditioning effects.

The ACE does not project directly to the trigeminal motor nucleus or the sensory nuclear complex. TAKEUCHI *et al.* (1988) revealed that the supratrigeminal region received projections from the ipsilateral ACE and projected heavily to the contralateral trigeminal motor nucleus. Therefore, the ACE modulation of the motor nucleus will be predominantly contralateral. However, the present study showed that the inhibitory effect of ipsilateral ACE stimulation was almost twice that of contralateral stimulation. This indicates that the modulatory effect is relayed to areas other than the supratrigeminal region. The ACE sends efferents to the ventromedial hypothalamic nucleus and lateral hypothalamic area (LHA) which connect reciprocally with the PAG and directly to the PAG (CONRAD and PFAFF, 1976; HOPKINS and HOLSTEGE, 1978; PITTMAN *et al.*, 1979; MORRELL *et al.*, 1981; PRICE and AMARAL, 1981). It is well known that the PAG exerts an inhibitory effect on the nociceptive neurons in the trigeminal nucleus caudalis through the RAM (SESSLE *et al.*, 1981). Therefore, the effect of ACE stimulation on the JOR appears to involve relays from other structures such as PAG and LHA.

This hypothesis is consistent with the reports that PAG conditioning stimulation inhibited the JOR (SESSLE *et al.*, 1981), and that the typical effect of hypothalamic conditioning stimulation on the JOR was inhibition (ACHARI and THEXTEN, 1972; LANDGREN and OLSSON, 1980). In addition, CARSTENS *et al.* (1983) reported that the activities of nociceptive neurons in spinal dorsal horn were inhibited by the LHA stimulation in the cat. Consequently, we hypothesized that the somatosensory information from the various levels of CNS converging onto ACE arouses the emotions, and these emotional changes modulate the trigeminal nociceptive reflex. However, as LEDOUX *et al.* (1988) have already stated that PAG are critically involved in the arrest of somatomotor activity, inhibition of the nociceptive reflex can be produced both at the level of the trigeminal motor nucleus as well as of the sensory nucleus. Determination of the site concerning the modulation of JOR by ACE will require further experimentation.

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