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The Nociceptive Jaw-Opening Reflex: Evidence for Alpha$_2$ Adrenoceptor Involvement

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CURTIS, A. L. AND J. MARWAH. *The nociceptive jaw-opening reflex: Evidence for alpha$_2$ adrenoceptor involvement.* PHARMACOL BIOCHEM BEHAV **26**(2) 437–444, 1987.—The ability of alpha adrenoceptor agonists to modulate the tooth pulp stimulation evoked (TPS) jaw-opening reflex (JOR) was investigated in rats and rabbits. Low doses of clonidine (6.25–50 μg/kg, IV) significantly increased dEMG thresholds. These effects were antagonized by alpha$_2$ adrenoceptor antagonists (e.g., yohimbine), but *not* by alpha$_1$ adrenoceptor antagonists (e.g., prazosin) or mu receptor antagonists (e.g., naloxone). Polar alpha$_2$ adrenoceptor agonists (e.g., ST-91 and 4-hydroxyclonidine) that cross the blood brain barrier (BBB) poorly and lipophilic alpha$_1$ adrenoceptor agonists (e.g., ST-587) that cross the BBB easily were without affect on the TPS-JOR. Structures of the peripheral efferent neurocircuitry of the JOR (e.g., the digastric muscle and the neuromuscular junction of the digastric muscle and its motor nerve, the mylohyoid) were shown *not* to be active sites of clonidine's effect on the TPS-JOR. Treatment with phentolamine (an alpha adrenoceptor antagonist that poorly crosses the BBB) completely antagonized clonidine's initial transient cardiovascular (pressor) effect without altering its TPS-JOR effects. Pretreatment with reserpine (a catecholamine depleting agent) failed to alter clonidine's affects on the TPS-JOR. Our studies suggest that alpha$_2$ adrenoceptors potently modulate the TPS-JOR and such modulation may be important in understanding trigeminal neuronal circuitries that partake in pain processing.

Alpha$_2$ adrenoceptors Alpha$_1$ adrenoceptors Clonidine Antinociception Jaw opening reflex
Mean arterial pressure Phentolamine Reserpine

IN addition to the prominent involvement of the opiatergic system, several studies have demonstrated a significant link between catecholamine neurotransmission and antinociception [1, 15, 27, 36, 39]. Unlike the other neuroreceptor systems, the extent to, or the manner in which adrenoceptors may be involved in the mediation of nociception has not been previously determined. Additionally, none of the reports to date have studied the effect of manipulating the catecholamine antinociceptive neurocircuitry on cardiovascular function. This is an important consideration since the catecholamine nervous system plays an important role in cardiovascular physiology.

In an attempt to address some of these issues, we have examined in both rats and rabbits the role of the alpha adrenoceptor system in a sensitive nociceptive paradigm, the tooth pulp stimulation (TPS)-evoked jaw-opening reflex (JOR). The JOR is an orofacial masticatory reflex that can be elicited by tooth pulp stimulation (pulpal-derived pain) and has been quantified by measurement of the digastricus muscle electromyogram (dEMG) [43]. The TPS-JOR is considered to be a presumed nociceptive assay system since: (a) the tooth pulp consists largely of small myelinated A delta fibers [2]; (b) tooth pulp fibers have a high threshold to electrical stimulation with conduction velocities in the A delta and C range; (c) digastricus muscle electromyogram is correlated with the onset and amplitude of the A delta activity; (d) tooth pulp stimulation parameters in the physiological range linearly affect the latency and magnitude of digastricus muscle activation [8, 9, 20, 22, 44]; (e) A delta fiber activity is correlated with pain intensity in human volunteers; and (f) the JOR is suppressed by stimulation of endogenous antinociceptive neuronal circuits (e.g., the periaqueductal gray and certain raphe nuclei) as well as by analgesic drugs [5, 8, 12, 32, 37, 43], but not by stimulation of non-antinociceptive central structures [32].

The present study describes several aspects of catecholamine modulation of the TPS-JOR (neuroreceptor(s), site(s) and mechanism(s) of action). We now report that activation of alpha$_2$ adrenoceptors in the JOR neurocircuitry elicits potent antinociceptive effects. These alpha$_2$ adrenoceptors are *not* located peripherally (the digastricus muscle or the neuromuscular junction) and there appears to be *no* direct physiological correlation between alpha$_2$ adrenoceptors that mediate cardiovascular function and those that partake in antinociception. Such data coupled with the lack of effects of polar alpha$_2$ adrenoceptor agonists and reserpine strongly

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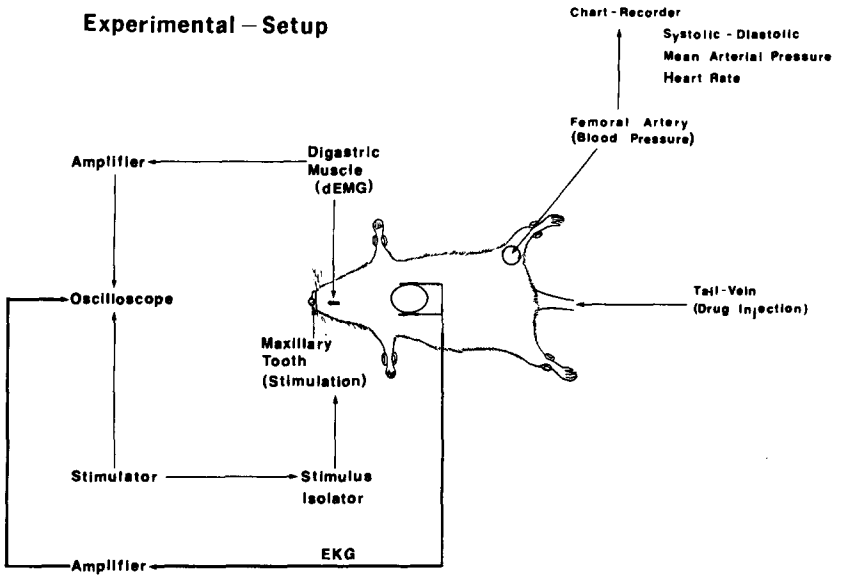

FIG. 1. Schematic of the basic experimental set-up for the rat. The same arrangement was used with the rabbit; however, drug injection was carried out through a cannulated medial vein of the rabbit ear.

suggests that the alpha$_2$ adrenoceptors modulating the TPS-JOR are most likely located postsynaptically in the central nervous system.

METHOD

Animals

This study involved male Sprague Dawley rats (200–390 g, Harlan Laboratories, Indianapolis, IN) and male albino rabbits (2–3 kg, Bunney Farms, Brazil, IN).

Anesthesia and Surgery

Animals were anesthetized with pentobarbital sodium (50 mg/kg); administered IP for rats and through a cannulated medial ear vein for rabbits. Animals were placed in a supine position on a heating pad for surgery and experimentation. The trachea was intubated and body temperature was monitored by a rectal thermistor and maintained by a heating pad at 37±1°C for rats and 39±1°C for rabbits. Depth of anesthesia was monitored by pupil size (response to light), heart rate (continuous electrocardiogram monitoring that displays heart rate), and respiratory condition (respiratory rate measured and displayed on a chart recorder). Surgery involved: the isolation of the left digastric muscle (one of the jaw openers) from its surrounding connective tissue and the exposure of the motor innervation to that muscle, the mylohyoid nerve; the exposure and ligation of the femoral nerve (at the point where it exits the peritoneal cavity) and exposure of the innervated quadriceps femoris; and cannulation of the femoral artery. An additional dose of pentobarbital (15 mg/kg, IV) was administered as a supplement 45 min before commencing experimentation. In previous studies, this anesthetic supplement has been shown to cause no appreciable effects on the TPS-JOR during the 100–120 minutes required for the study of drug actions [8].

Apparatus and Experimental Design

The jaw-opening reflex (JOR) was elicited by tooth pulp stimulation (TPS) and quantified by the digastricus electromyogram (dEMG). Our experimental design employed stimulation of the left maxillary tooth and recording the dEMG threshold (pain index). Bipolar stimulating electrodes (silver wires 0.1 mm diameter and insulated except for the tips) were implanted into the dental pulp of the left maxillary incisor. (See Fig. 1 for experimental setup.) Stimulation was provided by a Grass S88 stimulator/SIU-5 isolation unit (constant voltage) and quantified by an in-line voltmeter. Records of the TPS-evoked dEMG threshold were obtained by implanting bipolar silver needle electrodes (interpolar distance 2–3 mm) in the rostral-most aspect of the ipsilateral anterior belly of the digastric muscle. Amplification and filtering of the dEMG signal (DC recording 10× bandwidth: DC to 100 Hz) was achieved with a differential preamplifier and a storage oscilloscope. In some preparations the TPS consisted of trains of 1, 2, 4 and 8 rectangular pulses of 0.1 msec duration with an 0.8 msec interpulse delay. The train durations for 2, 4 and 8 pulses were 1, 2 and 4 msec respectively, presented at 1 Hz. The dEMG threshold was determined by gradually increasing the intensity of the stimulus until the first response could be detected in the amplified signal, and if that response was followed by five identical additional responses to the next five identical stimuli then that stimulus voltage value was recorded as the dEMG threshold. For all conditions and preparations in this study, 90 V was the "cut-off" intensity of TPS. Control readings were monitored at 5 minute intervals for 20 minutes.

Drug studies commenced only when minimal fluctuations (±4% of the first dEMG threshold value) in the thresholds for the dEMGs had been observed. All drug testing conditions consisted of an injection point followed by a 20 minute observation (data) period. Previous studies have shown that

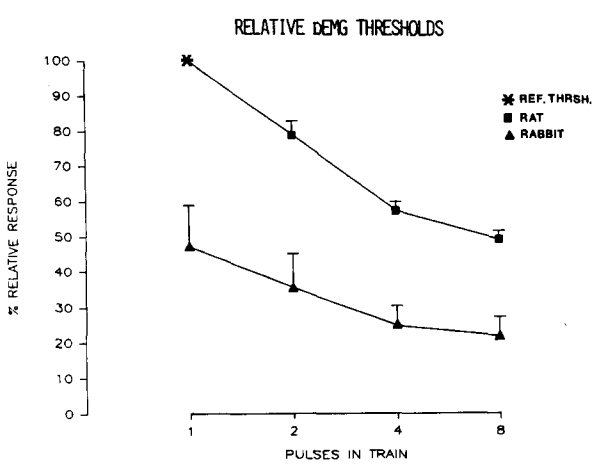

FIG. 2. The mean value of the single pulse TPS-evoked dEMG threshold in the rat (n=60) was used as the reference threshold (ref. thrsh.) to which all other control dEMG threshold values were compared. Note the inverse relationship between TPS pulse range (pulses in train) and the dEMG threshold response; and the preservation of this inverse relationship in the rabbit (n=5).

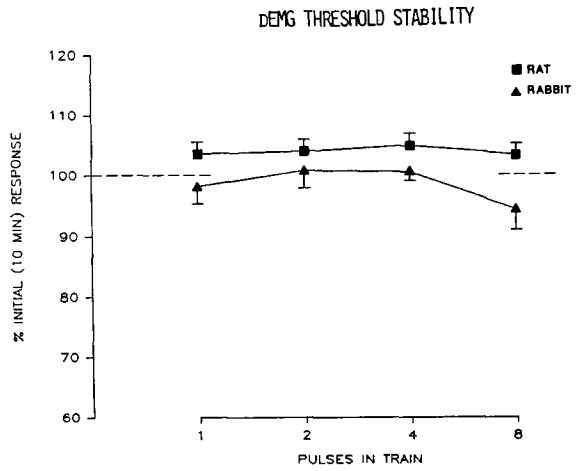

FIG. 3. The stability of the dEMG threshold responses elicited by the TPS pulse range (pulses in train) in rats (n=60). dEMG threshold values at 0, 5 and 10 minutes were the source of the initial control mean to which dEMG threshold values were compared for the next 30 minutes. There was no significant change of the dEMG threshold from the initial control mean (100% response). Readings were made at 5 minute intervals. All values were expressed as mean±SEM.

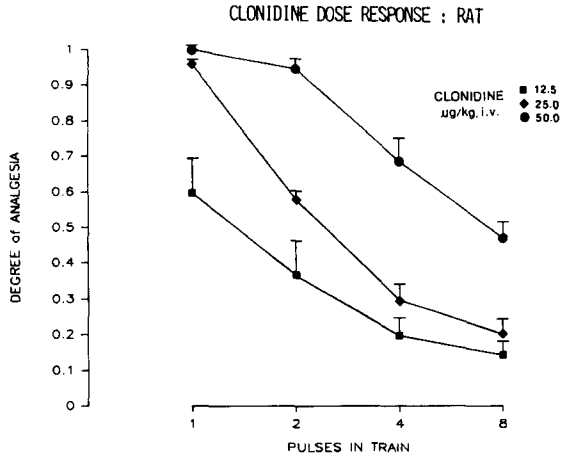

FIG. 4. The effect of a dose range of clonidine (12.5, n=7; 25, n=4; and 50, n=10 µg/kg, IV) on the degree of analgesia (DOA) in rats. A value of 1 on the DOA scale represents maximum analgesia. Control dEMG threshold values are normalized to zero.

the onset of the drug effect is evident within 30 seconds and usually peaks by 5 minutes [8].

Additional preparations were:

(1) Continuous measurement of pulsatile and mean arterial blood pressure (MAP; by femoral arterial cannulation) with a pressure transducer (Statham, P23Db), while simultaneously determining the dEMG threshold before, during and after clonidine administration in both the rat and rabbit.

(2) For determination of clonidine's effects on digastricus the following preparations were employed: (a) muscle isolation was achieved by direct topical application of d-tubocurare (dTC) to the exposed muscle; and (b) surgical isolation was achieved by cutting the motor nerve innervating the digastricus.

(3) The rat motor nerve innervating the digastricus, the mylohyoid, was isolated and used as a peripheral efferent site for mylohyoid nerve stimulation (MNS). MNS consisted of a single rectangular pulse of 0.1 msec duration at 1 Hz delivered by a monopolar electrode. In this manner in the same animal the TPS-evoked and MNS (intact)-evoked dEMG threshold were alternately determined at 5 minute intervals.

(4) In a different group of animals, in half of them the mylohyoid nerve was cut and in the other half the motor nerve was intact. The cut mylohyoid nerve group received the same monopolar single pulse stimulation to obtain a MNS (cut)-evoked dEMG threshold, while the intact half of this group experienced only TPS.

(5) Clonidine's effect upon another neuromuscular junction was determined by studying the drug's action on the EMG threshold of the quadriceps femoris muscle as elicited by stimulation of the ligated femoral nerve.

Drugs, Doses and Administration

Clonidine (CLD), a lipophilic alpha$_2$ adrenoceptor agonist (6.25, 12.5, 25, 50 and 100 µg/kg, IV); 4-Hydroxyclonidine (4-OHCLD), a non-lipophilic alpha$_2$ adrenoceptor agonist (200 µg/kg, IV); ST-91, a non-lipophilic alpha$_2$ adrenoceptor agonist (200 µg/kg, IV); ST-587, a lipophilic alpha$_1$ adrenoceptor agonist (200 µg/kg, IV); Yohimbine (YOH), an alpha$_2$ adrenoceptor antagonist (1 mg/kg, IV); Prazosin (PRAZ), an alpha$_1$ adrenoceptor antagonist (1 mg/kg, IV).

All of the above drugs were administered by a cannulated lateral tail vein (rats) or a cannulated ear vein (rabbits). The neuromuscular blocker, d-tubocurare (dTC; 10^{-4} mg/ml) was administered by topical application to the exposed digastricus. For pretreatment studies, phentolamine, a non-lipophilic alpha adrenoceptor antagonist (3 mg/kg, IV), was injected twenty minutes prior to the administration of clonidine; while a different group of animals received pretreatment with the catecholamine depleting drug, reserpine (10 mg/kg, IP) five hours before clonidine.

Clonidine, 4-hydroxyclonidine, ST-91 and ST-587 were gifts of Boehringer Ingelheim. Yohimbine, reserpine and

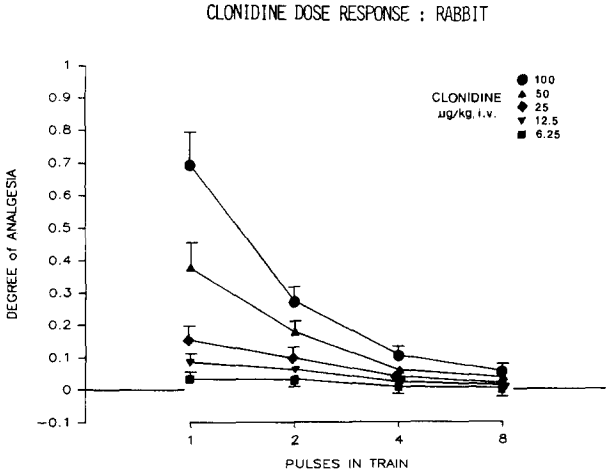

FIG. 5. The effect of a dose range of clonidine (6.25–100 µg/kg, IV) on DOA in rabbits (n=5 for all doses). All values are expressed as mean±SEM of DOA.

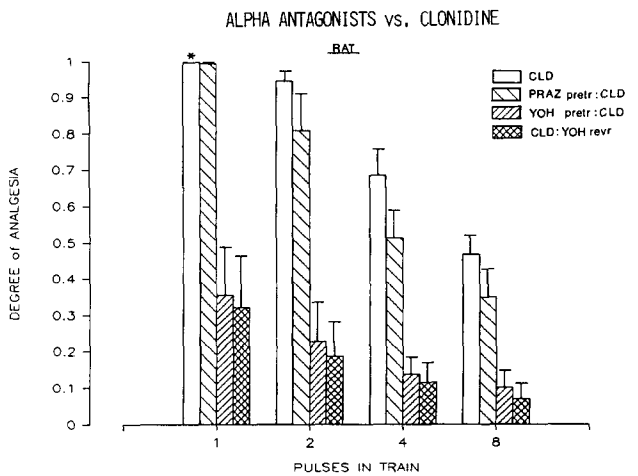

FIG. 6. The effects of various alpha adrenoceptor antagonists on the clonidine (50 µg/kg, IV; n=10) elicited DOA effect in the rat. The conditions are: prazosin pretreatment (PRAZ pretr; 1 mg/kg, IV; n=4; 20 minutes before); yohimbine pretreatment (YOH pretr; 1 mg/kg, IV; n=6; 20 minutes before); and yohimbine reversal (YOH revr; 1 mg/kg, IV; n=5; 20 minutes after). Competition at the alpha$_2$, but not alpha$_1$ adrenoceptor significantly ($p<0.05$) antagonized clonidine's effect. The observed antagonism was *not* order specific since the effects of YOH pretr and YOH revr were not significantly different. All values are expressed as mean±SEM of DOA. *Indicates an SEM of 0.0003.

d-tubocurare were purchased from Sigma. Prazosin was a gift of Pfizer.

Statistical Analysis

The data from this study was recorded as mean±SEM of either "degree of analgesia" or "percent control." The degree of analgesia was calculated as follows [27]:

$$\text{Degree of Analgesia} = \frac{\text{Post-Drug Value} - \text{Baseline Value}}{\text{Cut-off Value} - \text{Baseline Value}}$$

With this equation, a value of 1 represents maximum analgesia and a value of 0 represents no analgesia. Paired t-tests, Two Sample t-tests or ANOVA (one way) were used where appropriate. p-Values <0.05 were deemed statistically significant.

RESULTS

The experimental system employed for the rat is depicted in Fig. 1. A similar arrangement was used with the rabbit; however, drug administration was accomplished through a cannulated medial ear vein rather than a tail vein. Both the rat and rabbit preparations were stable for 8–10 hours.

Figure 2 shows the relationship between dEMG thresholds and pulse levels. The data for two, four and eight pulses are expressed relative to that of one pulse in the rat. These data demonstrate an inverse relationship between the dEMG threshold and pulse level. Additionally, these data also suggest that at a given pulse level the dEMG threshold is significantly lower for the rabbit as compared to the rat. To study the stability of the nociceptive (dEMG) thresholds, measurements were made every 5 min for 2–3 hours. As seen in Fig. 3, the dEMG responses at 30 min were not significantly different from the initial baseline readings taken at 0–10 min. This stability in response was observed at all pulse levels, i.e., one, two, four and eight pulses, for both rats and rabbits.

The dose-response effects of clonidine on dEMG threshold were determined for both rats and rabbits (Figs. 4 and 5). As seen from the figures, clonidine increased the dEMG thresholds in a dose-dependent manner. Two aspects of clonidine's effects on the dEMG threshold were observed: (a) the effects of clonidine on dEMG threshold were inversely related to the nociceptive stimuli (i.e., pulse levels)—at a given dose, clonidine was more effective in increasing dEMG threshold at the lower (less painful!) as compared to the higher (more painful!) pulses; and (b) the dEMG threshold was linearly related to the dose of clonidine, i.e., the higher the dose the larger the increase in the dEMG threshold.

To determine the subtype of the alpha adrenoceptor responsible for clonidine's effect on dEMG threshold, an alpha$_2$ (yohimbine) and an alpha$_1$ (prazosin) adrenoceptor selective antagonist were used. As seen in Fig. 6 treatment with yohimbine (1 mg/kg, IV; administered before or after clonidine) but not prazosin (1 mg/kg, IV) significantly antagonized the analgesic effects of clonidine (50 µg/kg, IV). The antagonists (yohimbine and prazosin) alone did not significantly alter dEMG thresholds, although, with yohimbine alone, there was a trend towards the development of hyperalgesia.

The effects of four alpha adrenoceptor agonists on TPS-evoked dEMG thresholds in the rat are shown in Fig. 7. For all drugs, the pre-drug baseline values are constant over time. Clonidine (CLD; a lipophilic alpha$_2$ adrenoceptor agonist) rapidly, reversibly and significantly elevated the dEMG threshold ($p<0.001$). Administration of non-lipophilic [4,39] alpha$_2$ adrenoceptor agonists (ST-91 or 4-hydroxyclonidine (4-OHCLD)), or the lipophilic [45] alpha$_1$ adrenoceptor agonist, ST-587, did not affect the dEMG threshold. The threshold altering effects of clonidine are significantly different from those of ST-91, 4-OHCLD or ST-587 ($p<0.001$). Since clonidine is an effective antihypertensive drug, it was also of interest to determine

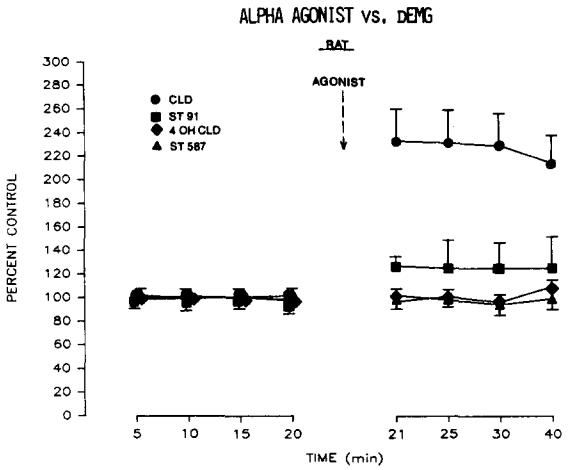

FIG. 7. The effects of various alpha adrenoceptor agonists on TPS evoked dEMG threshold in the rat. The alpha adrenoceptor agonists were: clonidine, a lipophilic alpha$_2$ agonist (CLD; 50 μg/kg, IV; n=10); ST-91, a non-lipophilic alpha$_2$ agonist (200 μg/kg, IV; n=4); 4-hydroxyclonidine, a non-lipophilic alpha$_2$ agonist (4-OHCLD; 200 μg/kg, IV; n=3); ST-587, a lipophilic alpha$_1$ agonist (200 μg/kg, IV; n=4). AGONIST indicates the point of administration of the alpha adrenoceptor agonist. Clonidine was the only agonist that significantly ($p<0.001$) elevated the dEMG threshold. All values are expressed as percent of mean control response±SEM. TPS was a 2 pulse stimulus at 1 Hz.

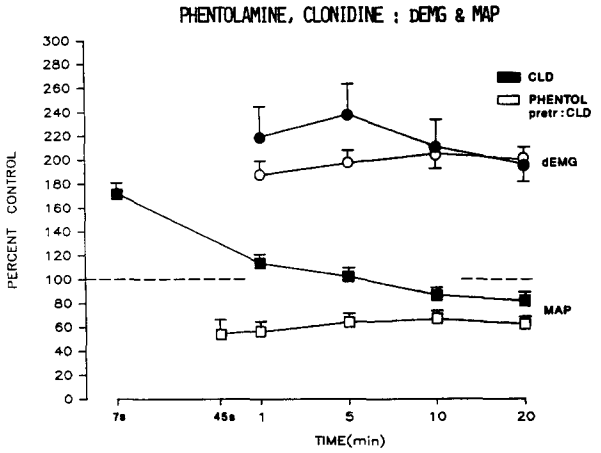

FIG. 8. Effects of clonidine (CLD; 50 μg/kg, IV; n=4) or phentolamine pretreatment followed by clonidine (PHENTOL pretr: CLD; PHENTOL at 3 mg/kg, IV 20 minutes before CLD at 50 μg/kg, IV; n=5) on the TPS-evoked dEMG threshold and simultaneously recorded MAP in rats. All values are expressed as percent of mean control response ±SEM. TPS was a 2 pulse stimulus at 1 Hz. s=seconds.

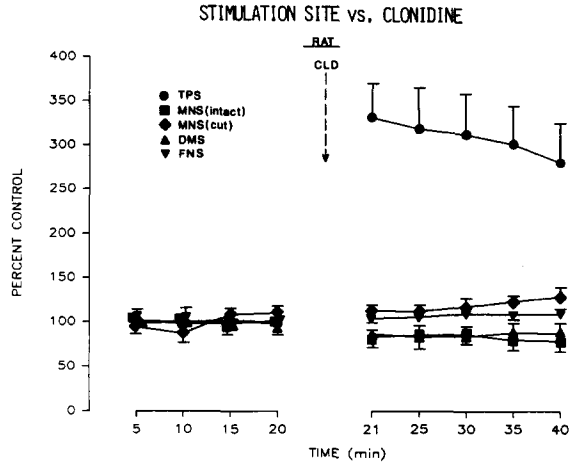

FIG. 9. The effects of clonidine (50 μg/kg, IV) on dEMG thresholds evoked by stimulation at various sites. Preparations: tooth pulp stimulation (TPS; n=5)-evoked dEMG threshold; intact mylohyoid nerve stimulation (MNS(intact); n=4)-evoked dEMG threshold; cut mylohyoid nerve stimulation (MNS(cut); n=5)-evoked dEMG threshold; digastric muscle stimulation (DMS; n=3)-evoked dEMG threshold; and ligated femoral nerve stimulation (FNS; n=11)-evoked quadriceps femoris EMG threshold. Stimulation of neurocircuitry that routes through the CNS was the only condition where the elicited EMG threshold response was significantly ($p<0.001$) elevated by clonidine. CLD indicates the point of clonidine administration. All values are expressed as percent of mean control response ±SEM. TPS was a 2 pulse stimulus at 1 Hz.

the effect of clonidine on simultaneously measured dEMG (nociceptive) thresholds and mean arterial pressure (MAP). As seen from Fig. 8, clonidine elicited a biphasic effect on MAP, an initial transient pressor phase lasting approximately one minute, followed by a sustained depressor phase (lasting 1–2 hours). The effect of clonidine on the dEMG threshold was essentially monophasic. There was a rapid (within one minute) increase in the dEMG threshold, which peaked at 5 minutes post clonidine injection. The effect on the dEMG threshold remained elevated for about 2 hours at values significantly greater than control. The magnitude of clonidine's effects on both MAP and dEMG threshold were dose related.

Figure 8 also depicts the effects of clonidine on MAP and TPS-evoked dEMG threshold, in phentolamine pretreated rats. As seen from the figure, the cardiovascular (pressor) effects of clonidine are completely antagonized by phentolamine (3 mg/kg, IV, 20 minutes before), whilst its antinociceptive effects remain intact. To determine whether clonidine directly affects neuromuscular transmission, its effects on evoked EMG thresholds were studied in several preparations (Fig. 9); (1) tooth pulp evoked dEMG; (2) dEMG as evoked by stimulation of the intact mylohyoid (motor) nerve; (3) dEMG as evoked by stimulation of the cut mylohyoid nerve; (4) dEMG as evoked by stimulation of the digastric muscle; and (5) EMG evoked in the quadriceps femoris muscle by stimulation of the femoral nerve. Clonidine significantly elevated dEMG threshold in the first case only, i.e., when the dEMG was evoked by TPS.

Figure 10 compares the effects of clonidine on dEMG threshold and MAP in control and reserpine pretreated (lesioned) rats. The effect of clonidine on dEMG is unaltered by reserpine pretreatment. However, reserpine pretreatment significantly enhanced the MAP (pressor) response to clonidine injection.

DISCUSSION

Electrical stimulation of the tooth pulp has been exten-

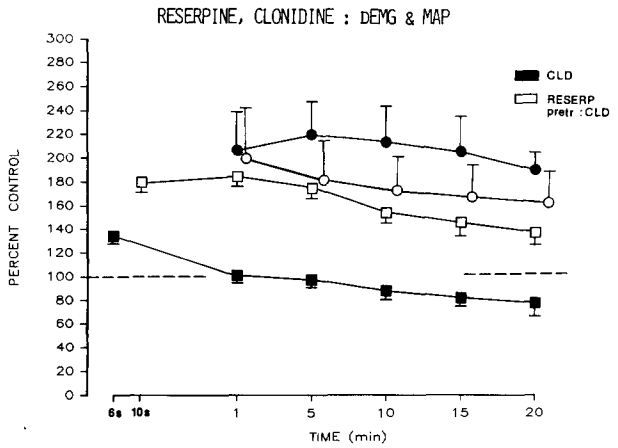

FIG. 10. Effects of clonidine (CLD; 50 μg/kg, IV; n=5) or reserpine pretreatment followed by clonidine (RESERP pretr: CLD; RESERP at 10 mg/kg, IP 5 hours before CLD at 50 μg/kg, IV; n=5) on the TPS-evoked dEMG threshold (round symbols) and simultaneously recorded MAP (square symbols) in rats. All values are expressed as percent of mean control response ±SEM. TPS was a 2 pulse stimulus at 1 Hz. s=seconds.

sively utilized as an analgesic assay in several species [5–9, 17, 30, 35, 38, 41, 47]. The present study has demonstrated a definite involvement of alpha adrenoceptors in the TPS-JOR. Currently, however, there exist two major schools of thought regarding the validity of the TPS-JOR as a nociceptive reflex. Evidence supporting the TPS-JOR as a nociceptive reflex is as follows: (1) the tooth pulp contains myelinated A delta fibers [2]; (2) tooth pulp fibers have a high threshold to electrical stimulation with conduction velocities in the A delta and C range. The JOR is readily elicited by stimulation of the tooth pulp and other high threshold afferent fibers within the distribution of the second and third divisions of the trigeminal nerve [11, 16, 25, 46]. The latency of the TPS-JOR indicates that the afferent arm of the reflex generally lies in the range of stimulus intensities which would excite a large proportion of myelinated afferent fibers [3]. Such findings suggest that the digastric reflex requires central summation, i.e., is more readily elicited by twin rather than single pulses [8]; (3) digastricus muscle electromyogram is correlated with the onset and amplitude of the A delta activity; (4) TPS parameters in the physiological range linearly affect the latency and magnitude of digastricus muscle activation [8, 9, 20, 22, 44]; (5) A delta fiber activity is correlated with pain intensity in human volunteers; (6) the TPS-JOR is suppressed by stimulation of endogenous antinociceptive neuronal circuits as well as by analgesic drugs [5, 8, 12, 32, 37, 43]; and (7) Mahan and Anderson [25] showed that endodontic therapy in cats eliminated the TPS-JOR indicating the response was not elicited by spread of current to extrapulpal nerve fibers.

It has also been reported that the TPS-JOR may not exclusively be a nociceptive reflex. Supporting this argument are findings that: (1) pulpal fiber velocities are also detected in the A beta range [3,31], with a high sensitivity to mechanical stimulation [10]; (2) threshold dEMG stimulation in awake animals does not result in escape or nocifensive behavior and in humans such stimulation elicits a "pricking" or an "aching" sensation but not pain [6, 10, 14, 23, 24, 28]; (3) Tal [42] showed that neonatal capsaicin treatment (which destroys unmyelinated C afferent fibers) in rats does not affect TPS-JOR. However, Holje *et al.* [19] reported that trigeminal neurons mediating pulpal pain are less affected by neonatal capsaicin treatment than nociceptive primary sensory neurons and C fibers at the spinal level; (4) McGrath *et al.* [24] showed that reflex activity in digastricus muscles can also be evoked by non-painful stimulation; and (5) it has been suggested that standard tooth pulp chamber stimulation in the rat incisor at current threshold for eliciting the JOR activity stimulates low threshold myelinated fibers in the surrounding periodontal (extradental) ligament as well as tooth pulp (intradental) afferent fibers [18,21].

Although tooth pulp-evoked reflexes may not universally be considered as objective measures of pain, nevertheless, at suprathreshold stimulus intensities nociceptive pathways certainly are involved. Moreover, at threshold levels these reflexes provide a valuable tool for studying trigeminal reflex mechanisms. Additionally, the TPS-JOR is relatively easily recorded and its threshold latency and magnitude readily quantified. Therefore, it seems to be an appropriate stimulus-response pathway with which to measure and assay potential analgesics. The reason for controversies surrounding the validity of the TPS-JOR as a nociceptive assay system might have to do with several variables: (1) route of administration of drugs; (2) animals species, if anatomical differences exist between different species then these might result in differences in current spread; and (3) the state of the animal or anesthetic may be responsible for the differences reported. Accepting the apparent sensory heterogeneity conveyed by dental pulp afferents however, does not necessarily invalidate the utilization of this test for assaying potential analgesics since the end points in many of the major antinociceptive assays (e.g., tail flick and hot plate) currently employed also are not exclusively elicited by nociceptive stimuli.

Our results indicate that in rats and rabbits the jaw-opening reflex elicited by tooth pulp stimulation is a sensitive experimental nociceptive paradigm for three reasons: (1) there is an inverse relationship between dEMG threshold and pulse level, i.e., higher pulse (more painful!) stimuli have a lower threshold; (2) at any pulse level there is no habituation or adaptation of response with repeated application of nociceptive stimuli; and (3) the dEMG threshold is linearly affected by analgesic dose.

In the current study, low doses of clonidine (6.25–50 μg/kg, IV) significantly increased dEMG thresholds in both rats and rabbits. These effects were inversely related to nociceptive intensity (i.e., the fewer the pulses in the train the greater the increase in dEMG threshold elicited by clonidine). Such antinociceptive effects of clonidine are most likely mediated by $alpha_2$ adrenoceptors, as pretreatment with yohimbine (an $alpha_2$ adrenoceptor antagonist), but not prazosin (an $alpha_1$ adrenoceptor antagonist), or naloxone (a mu receptor antagonist—data not shown) significantly antagonized these effects. Additionally, yohimbine did not antagonize the increase in dEMG thresholds elicited by administration of either morphine or pentobarbital (data not shown). The inability of prazosin or naloxone to antagonize the analgesic effects of clonidine could be construed as the inability of these antagonists to access the site(s) of action of clonidine. This possibility is unlikely, since previous studies have established that these compounds at the doses employed can not only rapidly access the central nervous system, but, in the case of naloxone also antagonize morphine's central actions [15,26]. For several reasons it appears that clonidine

most likely increases the tooth pulp stimulation (TPS) evoked digastricus electromyogram (dEMG) threshold by an interaction with centrally located alpha$_2$ adrenoceptors. First, 4-hydroxyclonidine and ST-91 have been shown to have a high affinity for alpha$_2$ adrenoceptors and in several nociceptive assays are devoid of analgesic activity when injected peripherally, but are active when injected centrally [4,39]. In the current study, the systemic administration of large doses (up to 200 μg/kg, IV) of 4-hydroxyclonidine or ST-91 failed to alter dEMG thresholds. Second, ST-587, an alpha$_1$ adrenoceptor agonist [45] was incapable of eliciting analgesia. Third, phentolamine, a non-lipophilic alpha adrenoceptor antagonist that crosses the blood brain barrier poorly [29], was incapable of antagonizing clonidine elicited analgesia. Fourth, clonidine was incapable of affecting the dEMG threshold when elicited by direct digastricus muscle stimulation or stimulation of the mylohyoid (motor) nerve.

The increase in dEMG threshold by clonidine is not correlated directly to the cardiovascular effects of this compound. For example, the effects of clonidine on MAP were biphasic, consisting of an initial transient pressor phase followed by a sustained depressor phase. During this sustained depressor phase the MAP generally decreased to values that were less than baseline controls. Conversely, during this same period, the nociceptive (dEMG) thresholds remained significantly elevated above baseline controls. Additionally, phentolamine, a predominantly peripheral alpha adrenoceptor antagonist, that completely blocked the transient cardiovascular effects of clonidine did not affect its antinociceptive actions. The central alpha$_2$ adrenoceptors responsible for clonidine elicited analgesia are most likely postsynaptically located. In reserpinized animals the antinociceptive effects of clonidine were unaffected, whilst its cardiovascular (pressor) effects were greatly enhanced. This regimen of reserpine has been previously shown to effectively deplete central presynaptic monoamine stores [13]. A likely candidate for the location of these alpha$_2$ adrenoceptors is the soma of motoneurons of the trigeminal system.

Finally, it should be pointed out that this is not the only purported analgesic assay system where alpha$_2$ adrenoceptor activation has been demonstrated to elicit analgesia. For example, clonidine has also been shown to have analgesic activity in the more commonly utilized nociceptive assay tests, e.g., the tail flick, the tail withdrawal, the hot plate and vocalization paradigms [15, 27, 33, 34, 39, 40]. Thus, evidence from a diverse group of studies strongly supports a major role for alpha$_2$ adrenoceptors in the modulation of pain.

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