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CORTICAL POTENTIALS EVOKED BY NOCICEPTIVE STIMULI: THEIR DEPRESSION BY A FACTOR RELEASED DURING SAPHENOUS NERVE STIMULATION IN THE RAT

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1 The amplitudes of the first positive and the first negative waves of cortical potentials evoked by electrical stimulation of the dental pulp in the rat were decreased following electrical stimulation of the peripheral cut end of the saphenous nerve.

2 This effect was greatly diminished when the stimulation of the saphenous nerve was performed with the saphenous or femoral veins ligated.

3 During stimulation of the saphenous nerve of a donor rat, the subcutaneous tissue in the area supplied by the nerve was perfused; when the perfusate (in which a permeability-increasing factor was detected) was injected intravenously into a recipient animal, a decrease in the amplitude of the evoked cortical potential of the recipient rat was also observed.

4 Intravenous injections of 5-hydroxytryptamine, histamine, bradykinin, prostaglandins or adenosine-triphosphate produced no effect on the evoked cortical potential, whereas large doses of acetylsalicylic acid caused a decrease.

5 It is suggested that a humoral factor, released during sensory nerve stimulation, may help to modulate the processing of afferent inputs from pain receptors.

Introduction

Wall & Sweet (1967) reported that stimulation of sensory nerves or roots supplying painful cutaneous areas led to a temporary abolition of pain in man. Stimulation of sensory nerves in the rat was shown by Jancsó, Jancsó-Gábor & Szolczányi (1967) to elicit an inflammatory response and they suggested that a neurohumor might be released from pain-sensitive nerve terminals and be responsible for the inflammation. Hamamura & Garcia Leme (1973) and Garcia Leme & Hamamura (1974) gave evidence for the release of a permeability-increasing substance during antidromic electrical stimulation of the saphenous nerve of the rat. This substance can be distinguished not only from histamine, 5-hydroxytryptamine, acetylcholine and catecholamines, as previously suggested by Jancsó and his group, but also from plasma kinins, substance P, prostaglandins, high molecular weight proteins and adenosine-triphosphate (ATP). In the present paper we have investigated the possibility that this substance might also affect cortical potentials evoked by nociceptive stimuli.

Methods

Male adult Sprague-Dawley (Holzman) rats (250-300 g) anaesthetized with pentobarbitone sodium (30 mg/kg) were used.

Recording of cortical potentials evoked by electrical stimulation of tooth pulp

Animals were firmly held in a stereotaxic apparatus (Kopf stereotaxic, model 1404) and a stainless steel wire electrode implanted in a hole drilled in one of the upper incisors. The dental pulp was stimulated via this electrode by means of a Grass S-4 electronic stimulator, the animal being grounded through one of the auricular pieces of the stereotaxic apparatus. Stimulation was by pulses of 5 ms duration, 0.5 Hz and of sufficient voltage (2 mV-2 V) to induce a well defined evoked potential in the cortical somatic I area. The intensity of the pulses was kept constant throughout each experiment. No defensive responses of the animals were observed. A silver wire ball-tipped electrode, in contact with the exposed contralateral brain surface, recorded the evoked

cortical potentials against a reference electrode placed on a neighbouring cortical zone. Such potentials were amplified and displayed on a storage cathode-ray oscilloscope (Tektronix 564-B). Preliminary experiments showed that the amplitude of the evoked potentials did not vary more than 15% from the initial values during a period of at least 4 h, provided that the following conditions were observed: (1) the EEG exhibited a spindling pattern with predominant theta frequency as detected by an EEG-Frequency Analyzer (Nihon-Kohden MAF-5); (2) the anaesthetic level was kept constant by supplying additional doses of sodium pentobarbitone (of 1.5 mg) whenever EEG desynchronization was apparent, as monitored by a recording polygraph (Nihon-Kohden, RM-85), or when movements of the animal were observed; (3) the respiratory rhythm was regular; (4) variations of the animals body temperature were minimized by maintaining a stable ambient temperature and keeping the animals on a heated (37°C) surface.

The superimposed records of ten evoked potentials were photographed as controls, at the end of an initial 30 min observation period. The animals were then submitted to experimental procedures and a similar series of evoked potentials photographed at various time intervals. Results are presented as percentage alterations from control values of the amplitude of the first positive and the first negative waves of the evoked cortical potentials.

Experimental procedures

The animals thus prepared were submitted to the following procedures:

(1) The saphenous nerve was stimulated electrically as described by Jancsó *et al.* (1967). Rectangular pulses were applied to the peripheral end of the sectioned nerve by means of a bipolar stainless steel electrode and an electronic Grass S-4 stimulator. The duration of the pulses was 20 ms, the frequency 10 Hz and the intensity 1, 2 or 4 V, the pulses being monitored on a cathode-ray oscilloscope (Hewlett-Packard, 130-C). The local effects of the stimulation were observed in the skin area supplied by the saphenous nerve as an exudation of Evans blue dye previously injected intravenously (20 mg/kg, 1% aqueous solution).

(2) Some rats were injected intravenously with perfusates from the subcutaneous tissue of the paw of another animal, collected before and during electrical stimulation (20 min) of the saphenous nerve to that paw. The perfusion was made by introducing a polyethylene tube (3 mm external and 2 mm internal diameter) into the subcutaneous space of the paw distal to the point of nerve stimulation. A narrower tube, connected to a reservoir containing Tyrode solution at a temperature between 5 and 10°C, was introduced into the larger one so that its tip protruded beyond the end of the larger tube. Thus, the perfusion fluid reached the subcutaneous space through the inner tube and was collected through the outer one. The flow was adjusted to 1 ml/10 minutes. Perfusions started 10 min before the electrical stimulation of the nerve (control sample) and continued for the 20 min period of stimulation (test samples). During the collection of the control sample all handling of the nerve was avoided. In the perfusates collected during nerve stimulation there was demonstrable permeability-increasing activity (Garcia Leme & Hamamura, 1974).

(3) Some rats were injected intravenously with the following agents which increase permeability: histamine, 5-hydroxytryptamine, bradykinin, ATP, prostaglandins E_1 and E_2 and acetylsalicylic acid.

Drugs used

Pentobarbitone sodium (Nembutal), Abbott; acetylsalicylic acid in 41% L-lysine solution (Endosprin), Enila; histamine diphosphate, Sigma; 5-hydroxytryptamine (Serotonin), Man Research; bradykinin (BRS-640), Sandoz; adenosine-triphosphate (ATP), Koch-Light; prostaglandins E_1 and E_2, Upjohn.

Table 1 Amplitude of cortical potentials evoked by dental pulp stimulation of control rats during 3 hours.

	Amplitude (% initial value)					
	5 min	*15 min*	*30 min*	*60 min*	*120 min*	*180 min*
First positive wave	99 ± 4.33	101 ± 3.63	95 ± 3.65	93 ± 3.10	99 ± 4.55	96 ± 3.98
First negative wave	100 ± 1.44	100 ± 1.37	100 ± 1.96	103 ± 2.13	103 ± 3.41	100 ± 2.71

Results are mean with s.e. from nine animals.

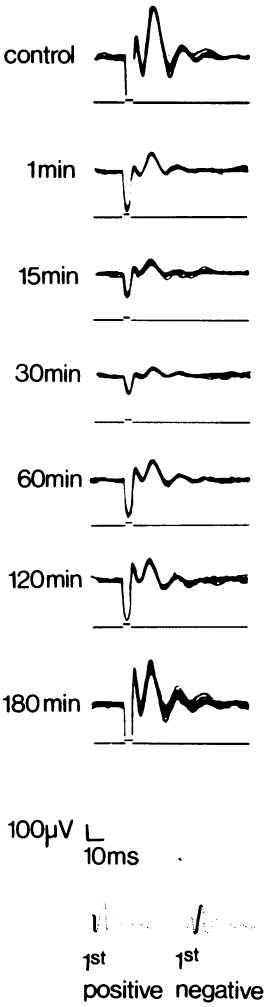

Fig. 1 Typical oscillographic recordings of 10 superimposed cortical potentials (upper trace) evoked by dental pulp stimulation (lower trace) in the rat. Control and a series of recordings obtained 1, 15, 30, 60, 120 and 180 min after a 20 min period of electrical stimulation (1V, 10 Hz) of the saphenous nerve of the animal. Note a progressive decrease in the amplitude of the first positive and first negative waves (negative up) of the evoked potential and a partial reversal in the last two records.

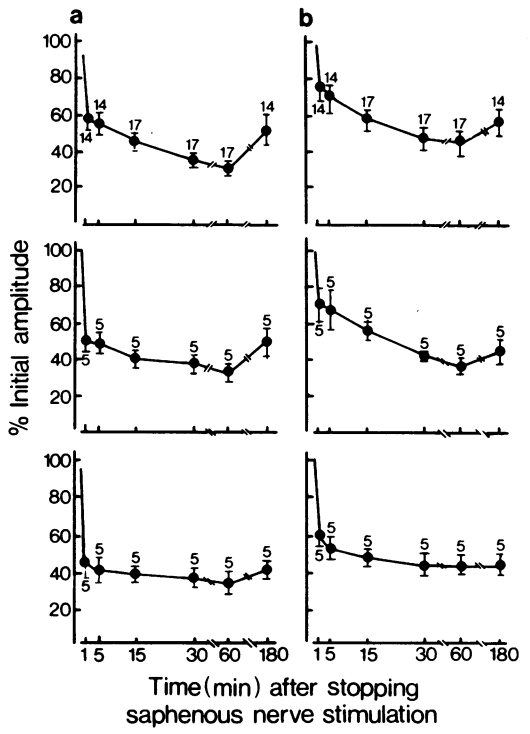

Fig. 2 Amplitude of (a) the first positive and (b) first negative waves of the cortical potentials, evoked by dental pulp stimulation, following a 20 min period of electrical stimulation (10 Hz) of the saphenous nerve with stimuli of various intensities: 1 V, 2 V and 4 V from top to bottom. Figures indicate number of animals in each experiment. Results are mean with s.e.

Results

The amplitudes of the first positive and first negative waves of cortical potentials evoked by electrical stimulation of the dental pulp in control animals during a period of 3 h are presented in Table 1. It should be noted that the potentials did not vary significantly from the initial values throughout the 3 hours. Stimulation of the saphenous nerve for 20 min with 1, 2 or 4 V caused a marked decrease in the amplitude of the first positive and first negative waves of the evoked cortical potentials which were measured from 1 min after stopping the stimulation of the nerve up to 3 hours. The waves decreased to a minimum between 30 and 60 min and partially reverted in the remaining observation period. No attempt was made to follow a complete reversal. Figure 1 shows a typical experiment. Figure 2 summarizes the results obtained.

Ligation of the saphenous or femoral veins, high in the thigh, prior to the stimulation of the saphenous nerve, greatly reduced the decrease observed in the first positive and first negative waves of the evoked cortical potentials. The first negative wave remained almost unaltered. The

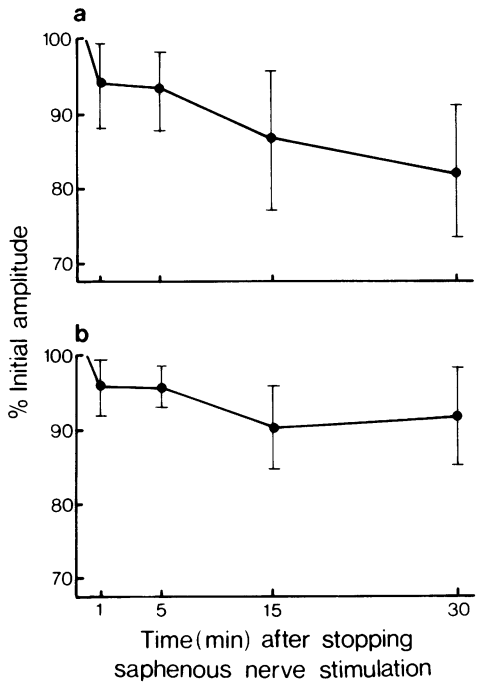

Fig. 3 Amplitude of (a) the first positive and (b) first negative waves of the cortical potentials evoked by dental pulp stimulation after a 20 min period of electrical stimulation (1 V, 10 Hz) of the saphenous nerve with saphenous or femoral veins ligated. Observe that the decrease in amplitude of the waves was much less than in Figure 2. Results are mean with s.e. of 5 experiments.

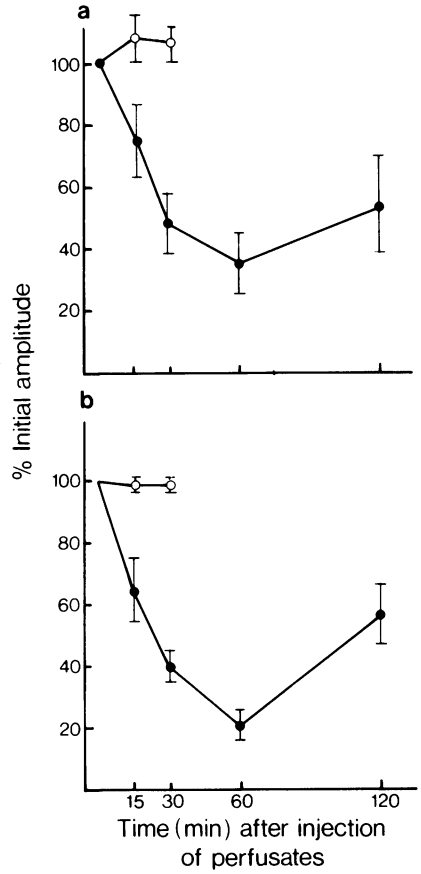

Fig. 4 Amplitude of (a) the first positive and (b) first negative waves of the cortical potentials evoked by dental pulp stimulation following intravenous injections of perfusates collected from the subcutaneous tissue of donor rats paws during 20 min electrical stimulation (1 V, 10 Hz) of the saphenous nerve. The volume of perfusate injected in each case was 1 ml. (•) perfusates obtained during the stimulation of the nerve; (○) perfusates obtained before the stimulation of the nerve. Results (mean with s.e.) were obtained in 14 recipient animals.

observations were carried out in 5 animals during 30 min (Figure 3).

Intravenous administration of perfusates (1.0 ml) collected from the subcutaneous tissue of donor rats in the area supplied by the saphenous nerve during its electrical stimulation, led to a decrease in the cortical potentials observed in the recipient animals. A marked decrease was observed 15 min after the injection which reached its minimal value at about 60 min, with a partial reversal thereafter. On the other hand, perfusates collected from non-stimulated paws of donor rats had no effect on the evoked cortical potentials of recipient animals. Figure 4 shows the results obtained in 14 recipient rats.

No effect on the amplitude of either component of the evoked cortical potential was observed within 30 min of the intravenous injection of various doses of histamine (0.5, 1 and 2 μg), 5-hydroxytryptamine (0.1, 0.2 and 0.4 μg), bradykinin (0.5, 1 and 2 μg), prostaglandin E_1 (0.1, 0.2 and 0.4 μg), prostaglandin E_2 (0.1, 0.2 and 0.4 μg) or ATP (0.1, 0.2 and 0.4 μg). Each dose was tested in at least five animals. Acetylsalicylic acid in doses of 100 and 200 mg/kg was ineffective but in a dose of 300 mg/kg it produced a decrease in the amplitude of the first positive and first negative waves of the evoked cortical potentials (Table 2), as already observed by Schmidt (1972). However, this decrease was less marked than those observed following stimulation of the saphenous nerve or injection of active perfusates.

Table 2 Amplitude of cortical potentials evoked by dental pulp stimulation of the rat following intravenous injections of acetylsalicylic acid.

	Evoked cortical potentials (% initial amplitude) at 3 times after drug injection					
	First positive wave			*First negative wave*		
	5 min	*15 min*	*30 min*	*5 min*	*15 min*	*30 min*
Acetylsalicylic Acid						
100 mg/kg	96 ± 3.33	94 ± 3.62	87 ± 4.31	98 ± 0.74	98 ± 1.32	96 ± 1.94
200 mg/kg	82 ± 3.98*	89 ± 7.40	86 ± 9.60	95 ± 3.25	96 ± 4.56	92 ± 4.96
300 mg/kg	77 ± 9.89*	67 ± 12.83*	65 ± 15.81*	92 ± 6.89	78 ± 10.17*	68 ± 11.04*

Results are means with s.e. from at least five animals. Significant differences are indicated.
*$P < 0.05$ (Student's t test, by comparison with control values in Table 1).

Discussion

Our results indicate that a decrease in the amplitude of the first positive and first negative waves of cortical potentials evoked by nociceptive stimulation of the dental pulp can be obtained either by prior electrical stimulation of the peripheral cut end of the saphenous nerve or by intravenous injections of perfusates collected from paws of donor animals during the electrical stimulation of the saphenous nerve.

Electrical stimulation of the saphenous nerve leads to an increased vascular permeability in the skin area supplied by the nerve (Jancsó *et al.*, 1967) and, if the subcutaneous tissue of the paw is perfused under these conditions, a permeability-increasing factor can be detected in the perfusates (Garcia Leme & Hamamura, 1974). We suggest that this permeability factor (or factors) may be responsible for the decrease in the amplitude of the component waves of cortical potentials evoked by nociceptive stimuli.

As we have used anaesthetized animals, no direct correlation can be drawn between this central effect and pain perception. However, it is interesting to note that Wall & Sweet (1967) reported temporary abolition of pain in man by electrical stimulation of the sensory nerve supplying painful cutaneous areas. Their observation combined with our results raise the possibility that a humoral factor, probably originating from sensory nerve endings, may help to modulate the processing of the afferent input from pain receptors. Such a mechanism would be in addition to those involving spinal and supra-spinal nervous integrations (Melzack & Wall, 1965; Reynolds, 1969; Mayer, Wolfle, Akil, Carder & Liebeskind, 1971; Schmidek, Fohanno, Ervin & Sweet, 1971; Lico, Hoffmann & Covian, 1974). This suggestion is reinforced by the fact that ligation of veins draining the area supplied by the saphenous nerve greatly reduced the decrease in the amplitude of the evoked potentials. Furthermore, acetylsalicylic acid (in large doses) was able to reduce the evoked potentials and its analgesic properties are unquestioned. In addition, phenacetin, amidopyrine and phenylbutazone were shown by Schmidt (1972) to depress components of cortical potentials elicited by tooth pulp irritation. Even so, it remains to be demonstrated whether a decrease in cortical potentials evoked by nociceptive stimuli runs parallel with a decrease in pain perception.

The active factor present in our perfusates was mainly characterized by its permeability-increasing activity. However, its central nervous effects, described in this paper, are unlikely to be dependent on a permeability-increasing activity, as potent vaso-active drugs such as histamine, 5-hydroxytryptamine, bradykinin, prostaglandins, and ATP were devoid of such an action.

Finally, though in our experiments intravenous injection of bradykinin produced no effect on evoked cortical potentials, its intracerebroventricular administration to rabbits in the same range of doses has been shown (Ribeiro & Rocha e Silva, 1973) to increase the nociceptive threshold for electrical stimulation of dental pulp.

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