stores of 5-hydroxytryptamine[17], in which circumstance the antinociceptive action of morphine would be reduced[18].

Whilst this explanation could contribute to the existence of cross tolerance which we now report between morphine and two other sympathomimetic antinociceptive agents, the situation is more complex. Whilst amphetamine will increase the 'turn-over' of 5-hydroxytryptamine[19] it has little effect on its overall concentration in contrast to the depleting action of fenfluramine[17,20,21]. In addition, whilst we have reported that 2-aminoindane influences the metabolism of 5-hydroxytryptamine, the drug does not affect its concentration in whole brain[22].

Zusammenfassung. Laboratoriumsmäuse wurden durch die Behandlung mit Morphium gegen die schmerzlindernde Wirkung dieser Droge immunisiert. Darauf folgte Nachbehandlung mit Amphetamin oder 2-Aminoindan. Die Vorbehandlung mit Morphium machte sowohl gegen die schmerzstillende Wirkung von Amphetamin als auch von 2-Aminoindan immun. Es wird eine ähnliche Grundlage der schmerzstillenden Wirkung dieser Drogen angenommen.

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A Study on the Peripheral Mediators of Dental Pain

The formation of kinins by the local activation of tissue proteases may contribute to the production of pain. The role of bradykinin (B) as a mediator of pain has been extensively studied in the skin[1] and viscera[2]. Recently, it was shown that prostaglandins (PG) may also contribute to the production of pain by sensitizing the pain receptors to other stimuli[3]. After the discovery of the blocking effect of aspirin-like drugs upon the biosynthesis of PG's[4], the mechanism of the analgesic effect of these drugs is understood.

The present study was undertaken to elucidate the role of the release of B- and PG-like material from the tooth pulp, due to the electrical stimulation of dentine, and its possible relation to dental pain.

Material and method. The adult dogs were anesthetized by sodium pentobarbital (30 mg/kg, i.v.) and chronic bipolar electrodes were implanted into the dentine of an upper canine tooth as described previously[5].

Sixteen chronically bipolar electrodes implanted dogs were anesthetized with sodium pentobarbital and the pulp of the same tooth was perfused with Krebs' solution through a stainless steel cannula inserted into the pulp, near the free margin of gum at a rate of 0.2 to 0.5 ml/min. The effluent was collected from the perforated tip of the tooth and continuously added to the isolated superfused, in cascade, rat duodenum (RD)[6], cat jejunum (CJ)[7] and rat stomach fundus strip (RSF)[8] previously prepared according to the method of VANE[9]. The test organs were superfused with 37 °C Kreb's solution, containing atropine

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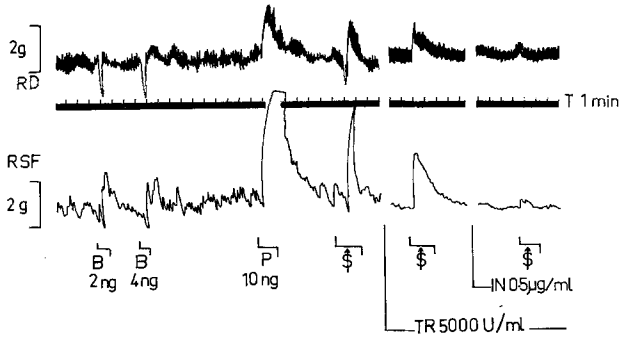

Fig. 1. Isolated, incascade, continuously superfused with Krebs' solution, rat duodenum (RD) and rat stomach fundus strip (RSF). Responses of both assay organs to directly applied bradykinin (B), PGE_2 (P) and to the effluent of pulp during the stimulation of dentine. (S). TR, Trasylol; IN, indomethacin.

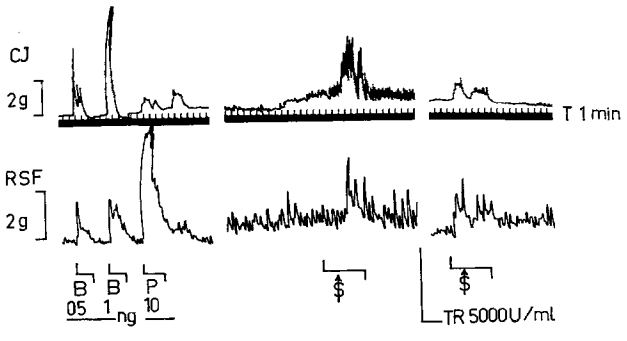

Fig. 2. Isolated, in cascade, continuously superfused with Krebs' solution, cat jejunum (CJ) and rat stomach fundus strip (RSF) to bradykinin (B), PGE_2 (P) and to the effluent of pulp during the stimulation of dentine (S). TR, Trasylol.

(1 µg/ml), mepyramine (1 µg/ml) and methysergide (0.5 µg/ml). CJ and RD were used for the identification of B[6,7] while RSF was used for the detection of PG's[10].

Isometric contractions of test organs were recorded by a Grass polygraph (Model 79 C) using force-displacement transducers (Grass FT. 03). Dentine was stimulated by voltages 5- to 10-fold of sensory threshold measured in conscious animals by square wave pulses of 0.5 msec duration and 10 cps frequency (Grass stimulator, M 58 C). The results were evaluated statistically using Student's *t*-test.

Results. The sensory threshold was determined and found to be 1.9 ± 0.4 (S.E., $n = 5$) volts, in conscious dogs. Superfusion of the effluent of tooth pulp over the assay organs did not induce significant responses. When dentine was electrically stimulated, it produced a biphasic response in RD and a contraction in RSF (Figure 1). B itself, caused a relaxation in RD and a contraction in RSF. PGE_2, however, produced a contractile response in both smooth muscle preparations. When Trasylol was added to the perfusion medium of the tooth pulp at the concentration of 5000 U/ml, the relaxation of RD and contraction of a CJ due to the stimulation of dentine was completely abolished, but the contraction of RD and RSF did not change (Figure 2). Further addition of indomethacin into the perfusion medium at the concentration of 0.5 µg/ml, caused a complete blockade in both assay organs. Neither Trasylol nor indomethacin at the concentrations used caused a change in response of assay organs to B and PGE-. The estimated amount of B-like material was found to be 2.0 ± 0.3 ng ($n = 5$) within 5 min stimulation of dentine while it was 4.5 ± 1.2 ng ($n = 5$) for PG-like material, as calculated depending on the responses of CJ and RSF to B and PGE_2.

Single i.v. injection of Trasylol at the dose of 10,000 U/kg caused an increase of sensory threshold in conscious dogs. This threshold was found to be raised to 4.0 ± 0.7 (S.E., $n = 5$) volts, in 6 min, and to 5.0 ± 0.5 (S.E., $n = 5$) volts, in 40 min after injection of Trasylol and gradually decreased and reached the control level within 20 min. Both measurements were significantly different compared with the control value ($p < 0.001$).

Intravenous injection of indomethacin at the dose of 2 mg/kg did not significantly change the sensory threshold compared with the control values. However, injection of Trasylol at the same dose to the indomethacin-pretreated (2 mg/kg i.v.) dogs caused a highly significant increase in pain threshold. In this group, the estimated value of threshold voltage measured 10 min after injection of Trasylol was found to be 8.7 ± 0.3 (S.E., $n = 5$) volts, a value which is significantly higher than that of obtained after injection of Trasylol alone ($p < 0.001$).

Discussion. The stimulation of dentine in conscious dogs induces a pain reaction[5,11,12], as well as a release of B- and PG-like material from the tooth pulp, according to the present results. It seems likely that the release of B- and PG-like material from the pulp due to the stimulation of dentine have an important role on the production of tooth pain, since both local hormones are known to be most potent pain-producers. Inhibition of B-sensitive assay organs, by the addition of Trasylol into the perfusion medium, indicates the possible activation of tissue kallikrein system due to the stimulation of dentine[13]. Again, inhibition of PG-sensitive assay organ by indomethacin indicates the release of PG-like material from tooth pulp.

In conscious dogs, Trasylol, but not indomethacin, significantly increases the sensory threshold induced by the stimulation of dentine. If the release of PG's is directly involved in the mediation of dental pain, then an increase in pain threshold due to the stimulation of dentine can be expected after indomethacin pretreatment. This is not the case according to the results of the present investigation. However, injection of indomethacin together with Trasylol caused a significant rise in sensory treshold compared with that of Trasylol alone. According to these findings, it seems unlikely that PG's directly act by stimulating sensory nerve endings. It perhaps sensitizes the nerve endings to B. Such a mechanism has been shown in man[3] and verified in dog spleen[14]. On the other hand, B causes the release of PG-like material from dog kidney[15] and dog spleen[14] and indomethacin can effectively reduce this release. It is still unknown whether or not B can cause the release of PG's in tooth pulp. The elaboration of B-like material from the tooth pulp due to electrical stimulation has recently been described by Inoki et al.[16] which further supports the present results.

Zusammenfassung. Mit In-vivo-Versuchen wurde an Hundezähnen gezeigt, dass auch in der Zahnpulpa durch elektrische Reizung des Dentins Bradykinin und Prostaglandin freigesetzt werden, wodurch auch in der Zahnpulpa die wahrscheinlichen peripheren Mediatoren für den Zahnschmerz ermittelt wurden.

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Zur Eindringtiefe dermal applizierter Wirkstoffe

Die kutane Applikation von Lösungen und Salben zur Therapie von Muskel-, Sehnen- und Gelenksaffektionen wird häufig angewendet und zeigt auch nicht selten Erfolge. Verschiedene Lösungsmittel werden benutzt, um die Penetration durch die Haut und die Permeation in tiefe Gewebslagen zu erleichtern. Wir haben kürzlich den Einfluss von DMSO auf das Eindringen von Phenylbutazon und Flumethason in die Tarsalgelenksflüssigkeit