effect was long-lasting, since, after the injection of 6-OH-DA for 1 to 4 weeks, the retinal sensitivity not only did not recover but decreased even more (data not shown). $Na_2S_2O_5$, an antioxidant, prevented the 6-OH-DA-induced effect and the preventive effect was also dose-dependent and quantitative. $Na_2S_2O_5$ itself had no effect on rhodopsin content and decreased the sensitivity only slightly. Since higher doses (up to 4 μmoles) of 2,4-dinitrophenol and H_2O_2 decreased the sensitivity only slightly, it appears neither uncoupled phosphorylation nor the cytotoxicity of H_2O_2 seem to be directly responsible for the effect of 6-OH-DA. On the other hand, 6-ADA and the *p*-quinone appear to be as effective as 6-OH-DA.

It has been long speculated that a conformation change in the photoreceptor protein (opsin) resulting from light stimulation exposes new active groups such as sulfhydryl[18], amino[19] and proton-binding groups[20]. It is thought that the regeneration of rhodopsin involves the covalent binding of 11-*cis* retinal to the ε-amino group of a lysine residue in the opsin[21,22] or through some form of a substituted aldimine linkage to the lysine amino and a cysteine sulfhydryl group[19]. *p*-Quinone, the primary and principal oxidation product of 6-OH-DA[5-7] could undergo covalent binding with these exposed free sulfhydryl and/or amino groups of opsin in the light-adapted retina, and thus prevent the regeneration of rhodopsin and concomitantly the recovery of retinal sensitivity. Interestingly, *p*-chloromercuribenzoate, a sulfhydryl-blocking agent, was similar to 6-OH-DA in its effect on retinal sensitivity and rhodopsin content.

The interaction between 6-OH-DA and rhodopsin was further supported by histological findings. In all the saline-injected controls (Figure a), the outer and inner segments of photoreceptors were clearly seen. In the 6-OH-DA (1 μmole) treated retina, the outer segments were damaged severely (Figure d) and partially (Figure c) 4 and 2 days, respectively, after injection. 1 day after injection, only part of the outer segments can be seen to be disorganized (Figure b), although functionally the sensitivity was already diminished. The rest of the retina seemed not to be affected.

Thus, the intravitreal administration of 6-OH-DA to light-adapted rats was found to prevent the recovery of retinal sensitivity and rhodopsin during the subsequent dark adaptation probably through the covalent binding of its oxidation product to the nucleophilic groups of opsin. The effect was dose-dependent, long-lasting, blocked by antioxidants, and non-specific to catecholaminergic neurons. Therefore, 6-OH-DA at doses comparable to that employed in this study should not be used to investigate the function of retinal dopamine. The interaction of 6-OH-DA with rhodopsin should be considered in the interpretation of results obtained even at lower doses.

Résumé. Nous avons observé que l'injection de la 6-hydroxydopamine dans l'humeur vitrée des rats adaptés à la lumière bloquait le rétablissement de la sensibilité rétinale pendant l'adaptation à l'obscurité subséquente au traitement. Il est possible que cette observation soit une conséquence de la réaction du produit de l'oxydation de la 6-hydroxydopamine avec les groupes nucléophiliques de la rhodopsine.

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Tolerance Development to the Antinociceptive Actions of Morphine, Amphetamine, Physostigmine and 2-Aminoindane in the Mouse

There have been many attempts to explain the actions of the narcotic analgesics in terms of selective interference with central chemical transmission[1,2]. However, recently several authors who had previously suggested that morphine selectively interferes with one transmitter system have advanced experimental work suggesting a far less specific mechanism of action[3,4]. This point has been highlighted by KUHAR, PERT and SNYDER[5] who were unable to demonstrate any obvious correlation between the distribution of opiate receptors in monkey brain with that of known neurotransmitters, and argued against an exclusive interference with the activity of cholinergic, serotoninergic or noradrenergic neurones.

In addition to the narcotic analgesics, a large number of other compounds possess antinociceptive activity including sympathomimetics (e.g. amphetamine) and cholinomimetics (e.g. physostigmine and oxotremorine). Our interest has been in trying to determine whether there is any correlation between the characteristics of narcotic analgesic activity and those of other agents which might indicate some similarity in mechanism of action.

Whilst the antagonism of the actions of morphine and its pharmacological analogues by the narcotic antagonists separates them from other antinociceptive agents indicating an action at a specific central receptor, there is evidence which connects the antinociceptive action of morphine with that of the sympathomimetics.

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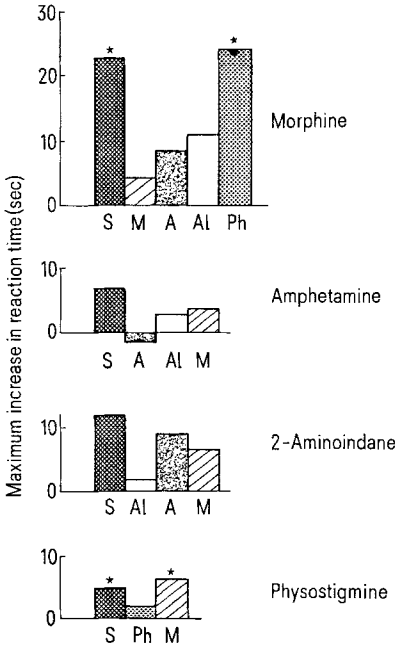
MAJOR and PLEUVRY[6,7] showed that pretreatment courses known to affect the concentrations of central transmitter substances had qualitatively the same influence on the antinociceptive actions of morphine and methylamphetamine in mice yet did not influence the latter's effect on stereotyped behaviour in the same manner. GORLITZ and FREY[8] showed that in rats pretreated with *p*-chlorophenylalanine the antinociceptive actions of morphine, amphetamine and *p*-chloro-amphetamine were all reduced, whilst their actions were enhanced in animals pretreated with 5-hydroxytryptophan. In addition PLEUVRY[9] showed that mice rendered tolerant to the antinociceptive action of morphine were also tolerant to that of methylamphetamine and vice versa.

Recent work in this Department has been concerned with the importance of the juxtaposition of the aromatic ring and the nitrogen atom in relation to the antinociceptive action of amphetamine, and the properties of compounds with structures intermediate between it and the narcotic analgesics will be the subject of a future paper (CROOKS, LITTLE and REES, in preparation). Amongst these compounds 2-amino-indane has been shown to be a more potent and more selective antinociceptive agent than is amphetamine. This present report describes the development of tolerance to its antinociceptive action, and explores the possibility of cross tolerance between it, morphine, amphetamine and physostigmine.

Methods. Groups of ASH/XP-strain female mice were used. The doses of drugs given were morphine hydrochloride (20 mg/kg^{-1}), 2-aminoindane hydrochloride (20 mg/kg^{-1})[10], (+)-amphetamine sulphate (20 mg/kg^{-1}) and physostigmine salicylate (0.1 mg/kg^{-1}). All injections were given by the i.p. route. Antinociceptive activity was measured by the Hot Plate Test[11]. In each case the reaction times were determined at 10 min intervals following injection, and these were compared with the reaction times of concurrently examined saline-treated mice.



The effects of morphine, amphetamine, 2-aminoindane and physostigmine on hot plate reaction time in groups of 12 mice. Mice were pretreated with saline (S, closed columns), morphine (M, hatched columns), amphetamine (A, shaded columns), 2-aminoindane (AI, open columns) or physostigmine (Ph, dotted columns). Stars above pairs of histograms indicate that there was no significant difference between the mean response ($P < 0.05$, Mann-Whitney 'U'-Test). In all other instances there were significant differences between the effects of each challenge drug in saline pretreated mice when compared with that in drug treated animals.

Groups of mice were pretreated with one of the above drugs at the stated dose 3 times each day (at 09.00, 13.00 and 17.30 h) for 5 consecutive days. Additional groups were similarly treated with saline.

The extent of tolerance development to morphine, amphetamine and 2-aminoindane was determined at the end of the pretreatment course by measuring the effects of each of the drugs in mice pretreated with the challenge drug, and in mice pretreated with either of the other compounds. In addition the effects of morphine were also determined in physostigmine-pretreated mice and that of physostigmine in morphine-pretreated mice. The reaction times of control mice challenged with saline were between 4 and 5 sec.

Results. The results are summarized in the Figure. The greater antinociceptive activity of 2-aminoindane compared to that of amphetamine at the same dose in saline-treated mice is evident.

Tolerance developed to the actions of all 4 drugs. Mutual cross tolerance was seen in any comparison between morphine, amphetamine and 2-aminoindane. No cross tolerance was apparent when the effects of morphine were examined in physostigmine-pretreated mice and vice versa.

Discussion. The existence of cross tolerance between morphine and amphetamine is compatible with the previous observation of cross tolerance between morphine and methylamphetamine[9]. Cross tolerance between either drug and the stereochemically more rigid sympathomimetic 2-aminoindane is now reported.

That this is not a non-specific phenomenon is apparent in the absence of cross tolerance between morphine and a cholinomimetic antinociceptive agent. In addition it is unlikely that reciprocal induction of microsomal enzymes is responsible for the phenomenon since neither morphine nor amphetamine displays this property to any significant extent[12–15].

Recently DUNCAN and SPENCER[16] reported that the sympathomimetic fenfluramine had demonstrable antinociceptive activity in mice, which in combination with morphine effected an enhanced response which they interpreted as potentiation. However, in mice pretreated with fenfluramine the antinociceptive action of morphine was abolished. They interpreted this phenomenon in terms of fenfluramine pretreatment depleting

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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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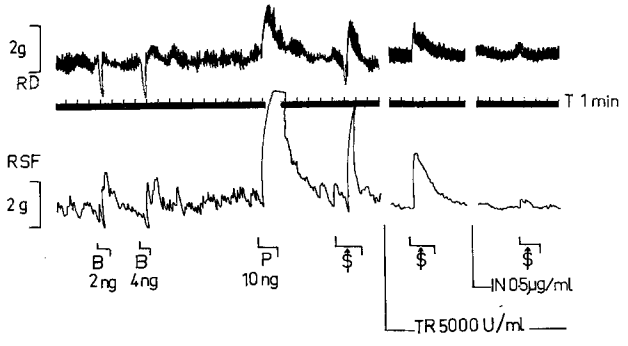

Fig. 1. Isolated, incascade, continuously superfused with Krebs' solution, rat duodenum (RD) and rat stomach fundus strip (RSF). Responses of both assay organs to directly applied bradykinin (B), PGE_2 (P) and to the effluent of pulp during the stimulation of dentine. (S). TR, Trasylol; IN, indomethacin.

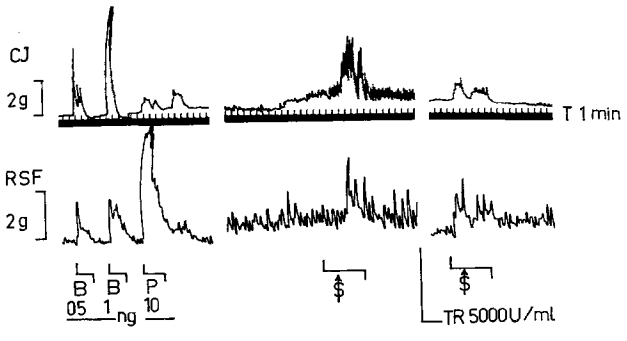

Fig. 2. Isolated, in cascade, continuously superfused with Krebs' solution, cat jejunum (CJ) and rat stomach fundus strip (RSF) to bradykinin (B), PGE_2 (P) and to the effluent of pulp during the stimulation of dentine (S). TR, Trasylol.