this work, the structure is solved by using the space group $C2/m$, and the atomic coordinates are nearly equal to the average values of the corresponding quantities of the two molecules A and B.

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2. Crystallographic data are in agreement with those reported by M. P. Gupta and W. H. Barnes, *Can. J. Chem.* **34**, 563 (1956).
3. In these cases, three oxygen atoms of the same kind, but from different asymmetric units, are in contact with the same K^+.
4. I thank Professor D. Harker for enlightening discussions. Financial support by NIH-A-3942 and NSF-GB-4056 is gratefully acknowledged. A complete description of the structure determination is in preparation.

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Aspirin: Action on Receptor in the Tooth

Abstract. *Impulse frequency recorded from dentinal receptors in the cat's tooth provided an indication of sensory excitation. Topical application of aspirin to the dentin promptly inhibited both steady-state discharge and response to a brief heat stimulus. When the drug was washed out, the initial excitability was restored in 15 minutes.*

The control or reduction of pain through ingestion of aspirin is a common observation, but its site of action has long been a matter of debate. If its action were primarily peripheral, it would be expected to decrease the excitability of the receptor structure which originates the "pain" signal or to increase selectively the threshold of those fibers which conduct nerve impulses from such receptors. Recent study of the dentinal receptor in the tooth has made possible the clear demonstration of the action of aspirin on the peripheral receptor.

The sensation aroused by excitation of the tooth with thermal, tactile, or chemical stimuli has been described by many investigators as primarily painful in character. Although pain may mask other modalities present in lesser degree, there is little evidence to support such a concept and there is little doubt that pain is the primary conscious response. The electron-microscopic study of the ultrastructure of a gradually tapering sensory nerve terminal located within the dentinal canal has been described by Frank (*1*), who observed the terminals to extend beyond the predentin and for short distances into the calcified portion of the dentinal tubule in human teeth. Our electrophysiologic evidence indicates the presence of single receptors distributed infrequently in the dentin of the canine teeth of cats, which respond to stimulation by heat, cold, distortion, and certain chemical agents (*2*). The fact that the electrical signals from these receptors could be obtained only when the recording electrode was between 0.15 and 0.20 mm from the pulp strongly suggests an identity between these units and those described by Frank. Reflex aversive movements of the tongue could be elicited by moderate stimulation of these receptors involving any one of these methods. Stimulation of these receptors probably gives rise to the sensation of pain in the animal.

Experiments were conducted on the canine teeth of cats, whose sensory axons follow the axis of the pulp and, turning abruptly, reach the odontoblast layer, where they branch extensively. Such a terminal branch may then enter a dentinal canal (*1*). No great differences exist in the sensory innervation of mammalian teeth except for those which are continuously erupting, as in rodents. Two cavities were prepared on the lateral aspect of the tooth to overlie the pulp and were separated by 2 to 5 mm. The cavity nearest to the incisal tip was placed in the close vicinity of the pulpal horn where the maximum number of receptors had previously been demonstrated by microapplication of acetylcholine (*3*). Records obtained from silver–silver chloride saline agar electrodes placed in such cavities have shown potentials originating from one to three receptors (*2*, *4*), this result being attributable to the fact that all dentinal receptors are located within dentinal tubules.

Amplification of these potentials by a Grass P-6 preamplifier, followed by a trigger circuit and an Atomic rate meter, provided a continuous record of discharge frequency while visual presentation and photography of the amplified impulse on a Tektronix 565 oscilloscope monitored the identity of the discharging unit. The frequency of impulse discharge is closely related to temperature (*2*). Therefore, the temperature of the bottom of the cavity nearest to the receptor was continuously recorded, and the ambient temperature was maintained by a heat lamp directed at the oral cavity.

This method (*3*) provides a continuous comparison of receptor temperature and discharge frequency. Thermal stimuli consisting of pulses of heat lasting 10 seconds were delivered at intervals by a coil of fine Nichrome wire, 1 mm in diameter, placed 2 mm from the surface of the tooth opposite the incisal electrode. Such pulses raised the temperature of the receptor several degrees Celsius and increased the discharge frequency to more than double its resting value, thus providing a quantitative indication of the irritability of the receptor. Aspirin was applied directly to the incisal cavity in droplets (1 μl) containing 40 ng of unhydrolyzed aspirin in normal Ringer solution.

The steady-state discharge of the dentinal receptor is influenced by its temperature (normal being approximately 34°C). If constant temperature is maintained during cavity preparation with a constant flow of Ringer solution at 39°C over the bur (to offset evaporative cooling), a steady discharge of 10 per second is most frequently obtained, but higher or lower frequencies are not infrequent, depending on conditions of preparation. Since my study concerned a depression of receptor response, a higher initial frequency was desired, and this was obtained by the addition of occasional small (1 μl) single drops of isotonic sodium citrate. The irritability of the receptor as a function of its initial frequency does not distinguish between receptors which have been bathed only with Ringer and those to which a few small drops of citrate have been added to increase their resting excitability.

In a typical experiment (Fig. 1) a steady-state discharge frequency of 20 to 30 per second was obtained, and a 10-second heat pulse was administered to provide a measure of the irritability of the receptor. The maximum frequency observed after the heat stimulus was 73 per second. For the next 2 minutes, both temperature and frequency returned toward their initial values.

At the end of this period, 1 μl of aspirin solution was applied to the incisal cavity between the plastic capillary of the electrode and the dentinal wall. The evaporative cooling resulting from this droplet reduced the temperature to approximately its initial value. The receptor discharge responded to the as-

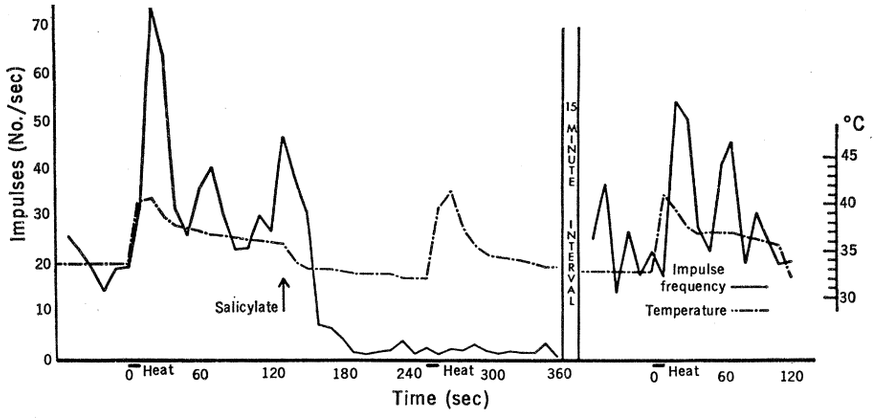

Fig. 1. Depression of receptor excitability by salicylates. Heat pulses were 10 seconds long, applied as indicated by solid bars. Salicylates were applied to cavity at arrow.

pirin application with a short burst (lasting 20 seconds, probably spontaneous) reaching a maximum of 47 per second after which the frequency fell rapidly and within 60 seconds reached 3 per second where it remained. Two minutes after application of aspirin, a heat stimulus (identical to the first) again resulted in an increase of temperature to 41°C, but no increase occurred in the discharge frequency. Subsequently, the temperature returned to its resting value (33°C), but there was no change in the frequency.

To establish the reversibility of this action on the receptor, I replaced the aspirin solution in the cavity with Ringer for 15 minutes after the last observation. Both temperature and frequency returned very closely to the control values at the start of the experiment. A third, identical, heat stimulus was applied to test the excitability of the receptor; the resulting maximum temperature was 41°C, as before, and the discharge frequency rose to 53 per second, which rate approached the value in the initial heat test. The final steady-state values, obtained 2 minutes later, were similar to those previously observed: temperature, 33°C; frequency, 23 per second.

These results indicate that the excitability of this receptor had very closely returned to its initial level in the 19 minutes following application of aspirin. The sequence of observations was subsequently repeated on the same receptor with similar results.

This typical experiment demonstrates the constancy of the single-receptor steady-state activity, and its excitability when tested with heat pulses reflected an equilibrium in its microenvironment, especially in the capillary blood flow in its immediate vicinity (*5*). This showed the prompt and profound reduction in the receptor excitability after topical application of 150 ng of aspirin in Ringer solution.

The constant effect of the test heat pulse in raising the temperature of the receptor's environment to 41°C in all three instances speaks against any marked change in the microcirculation, due either to systemic changes of the preparation or to the application of aspirin. Furthermore, although the steady-state frequency showed small variations, the four mean values obtained when aspirin was not present were 20, 26, 20, and 23 per second. These values relate to the receptor temperature (Fig. 1). Application of aspirin solution did not reduce the receptor temperature below 32°C, which corresponded to a frequency of 20 per second in the untreated tooth. Except for the possible transient excitatory effect for a few seconds after application of aspirin, the resulting changes in excitability cannot be attributed to thermal effects.

Although the discharge frequency of the receptor was not significantly reduced until 30 seconds after application of aspirin solution (Fig. 1), the activity of the receptor in other preparations has been depressed in as little as 10 seconds after application. Such short times suggest a direct action of the drug on the receptor, which is delayed only by the time necessary for the solution to flow through the dentinal tubule to the receptor.

The complex hydraulic forces present within the dentin reflect its structure and its relation to the highly vascular pulp. The normal peripheral closure of the dentinal tubules by the intact enamel serves to maintain a positive pressure (20 to 30 mm-Hg) of fluid resembling serum ultrafiltrate (*6*). On the other hand, if the peripheral end of the tubules is uncapped by mechanical removal of the enamel in the preparation of a cavity, the fluid balance is reversed because of the strong capillary forces within the tubule, and fluid may be rapidly absorbed by the dentin from the floor of such a cavity. The rate of flow within the tubule will vary according to the dimensions of the extracellular space, which fact accounts for the observed variation in latency of the effect of aspirin.

The observed reversibility of the inhibition by aspirin would be expected from the flushing of the tubule by the Ringer solution which was maintained in the incisal cavity during the 15-minute interval. It is unlikely that a significant chemical change in the aspirin took place in this interval since its rate of hydrolysis is 4 percent per hour at body temperature. However, when the aspirin solution was made up 24 hours before application, it was without effect on receptor excitability. By comparison, direct application of 1 μl of a 0.05 percent solution of Xylocaine to a similar cavity (*7*) usually required 10 minutes to completely inhibit spontaneous firing, and resulted in a reduction to half of the heat-evoked response.

Lim *et al.* (*8*) examined the reflex response to pain in the cross-perfused but neurally intact spleen of the dog when bradykinin was given by close injection into the splenic artery. This effect was blocked by aspirin only when injected into the donor dog in dosages resulting in plasma concentrations of 15 mg per 100 ml of blood, a probable peripheral site of action. No blocking effect has been found by these authors or by me when aspirin has been applied to segments of peripheral nerve and its capacity to conduct electrically evoked impulses is tested.

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