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EFFECT OF TEMPERATURE UPON THE CONFORMATION OF ACETYLCHOLINE RECEPTORS

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The use of irreversible blocking agents such as dibenamine (1) or benzilylcholine mustard (2) as tools to study the nature of drug receptors still remains a controversial subject in the field of molecular pharmacology. However, these agents have been employed to investigate the effects of temperature changes on alternative macromolecular geometries or transitions which may be relevant to their drug specificity.

Recent studies by Kunos and Szentivanyi (3), Buckley and Jordan (4), and Kunos et al. (5) have indicated that either the β-adrenergic receptors in the isolated frog heart act like α-adrenergic receptors at low temperatures or that there are two pools of adrenergic receptors, the availability of either being governed by temperature. However, Reinhardt et al.(6) observed that, on the isolated guinea pig atria, the affinity of β-blockers was not influenced by temperature. More recently, Harri (7) has demonstrated that the affinity of α-blockers on the adrenoreceptors of the toad heart is increased at low temperatures, while the affinity of the β-blockers is increased at high temperatures.

It has been observed (8,9) that the release of acetylcholine at neuromuscular junctions is a function of temperature. Taylor (8) reported that the output of acetylcholine is about 80 per cent lower at 20° than at 37° in the rat phrenic nerve diaphragm preparation. Furthermore, Ehrenpreis and Rosen (10) have demonstrated that the receptors involved in neuromuscular contraction undergo molecular conformational changes as a function of temperature. Like the earlier work of Taylor (8), the authors found a negative temperature dependence of blockade using an irreversible neuromuscular blocking agent.

The significance of investigating muscarinic acetylcholine receptors using irreversible blocking agents becomes apparent when one considers that k_2 is temperature dependent. Here,

$$D + R \underset{k_2}{\overset{k_1}{\rightleftarrows}} D\text{-}R$$

D is the drug concentration, R is the number of receptors available for binding, and D-R is the concentration of drug-receptor complex. With this in mind, we prepared a series of muscarinic blocking agents. These compounds were conceived and prepared on the principle of using an established pharmacologically active molecule as the carrier (a furan ring) for

a potential alkylating moiety (β-haloethylamine).

In our continuing study of the cholinergic receptors, we have taken the opportunity to study the effect of temperature changes on the binding of muscarinic antagonists on the guinea pig ileum. We report, herein, that at 21° the number of receptors available for binding is less than at 37°.

The irreversible muscarinic blocking agent, 2-[(2-chloroethyl)methylamino]ethyl 5-methyl-2-furoate hydrochloride, was prepared according to the method of Rosen et al.(11).

The guinea pig ileum was prepared by conventional methods (12,13). Contractions of the ileum were measured isotonically with a Harvard heart/smooth muscle transducer under a tension of 1 g. Contractions were elicited at equilibrium by injecting acetylcholine into the tissue bath every 2 min.

In a typical experiment, a dose-response curve was generated using acetylcholine injected at various concentrations. The $[A_{50}]$ of acetylcholine was used as the control dose for the remainder of the experiment. At this point, a concentrated solution of the irreversible blocker was added to the Tyrode's bathing solution such that a final concentration of 10^{-6} M of the inhibitor was obtained. Then, the $[A_{50}]$ of acetylcholine was injected, in the presence of the inhibitor, every 2 min for a 30 min exposure. After that, the tissue was washed with plain Tyrode's solution for an additional 30 min continuing the schedule of acetylcholine injections, and the height of muscle contraction was compared with that of control.

With a new tissue, an initial dose-response curve was obtained at 37°. Then, the temperature of the bathing solution was lowered to 21° over a 10 min period of time. After equilibrium had been established, a new dose-response curve was generated. The tissue was then subjected to the irreversible blocker (10^{-6} M), and the ability to block the effects of acetylcholine was determined. After removal of the drug from the bathing medium and continual washing for an additional 30 min, the temperature of the tissue bath was slowly raised to 37°, at which point the dose-response curve was repeated.

We have established that, like other blocking agents of the β-haloethylamine type, the formation of the aziridinium ring is rapid, and it is this moiety which is responsible for the alkylation of the receptors. We have observed that the $T_{1/2}$ for the aziridinium ion formed from 2-[(2-chloroethyl)methylamino]ethyl 5-methyl-2-furoate is 50 min at 30°. At 37°, the irreversible inhibitor at 10^{-6} M blocked 85 per cent of the muscular activity of the guinea pig ileum, as elicited by acetylcholine. This blockade could not be reversed by exhaustive washing for an additional 30 min (Fig. 1).

In studying the effect of temperature upon availability of a drug to be bound to a receptor complex, we initially generated a dose-response curve at 37° for acetylcholine. Then the temperature of the tissue bath was lowered to 21°. After equilibrium was reached, we observed that the response of the tissue to acetylcholine decreased to approximately 30

per cent of control (at 37°). This shift in dose-response mimics that obtained when the tissue is subjected to a competitive inhibitor in the presence of acetylcholine at 37°. This temperature effect upon muscle contraction was reversible in that, when the temperature was raised to 37°, the initial dose-response was obtained. Subjecting the tissue at 21° to 10^{-6} M of the irreversible inhibitor for 30 min eliminated essentially all contractual response. After washing the tissue for an additional 30 min at 21°, there was still no response of the smooth muscle to injections of acetylcholine. However, upon raising the temperature of the bathing medium to 37°, the response to acetylcholine returned to the level of initial control (at 37° - note Fig. 2). This indicated that, during the time of reaction at 21°, only a small fraction of the receptors had reacted with the irreversible antagonist, but this was sufficient to produce essentially a complete block at this temperature.

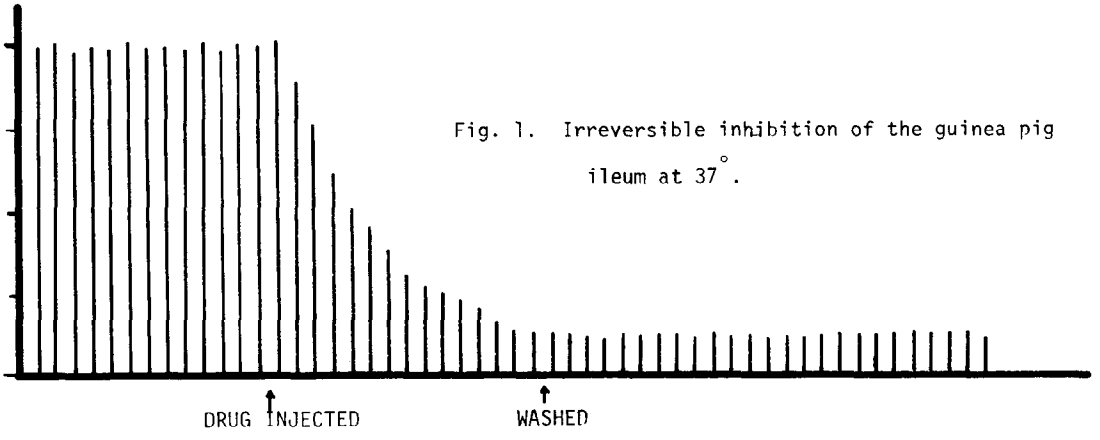

Fig. 1. Irreversible inhibition of the guinea pig ileum at 37°.

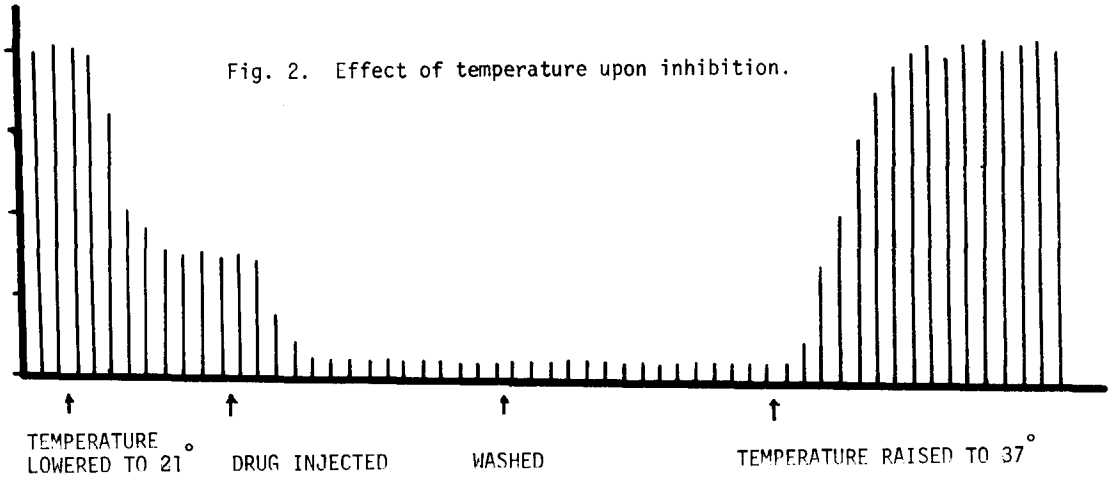

Fig. 2. Effect of temperature upon inhibition.

It is possible that, when raising the temperature to 37°, reversal of the block was due to cleavage of the drug-receptor bond. To test this hypothesis, the degree of reversal was determined when the temperature was raised from 21 to 37° and then lowered to 21°. The two curves were almost identical (Fig. 3). Thus, the reversal of the block produced by raising

the temperature was not due to a change in receptor occupancy.

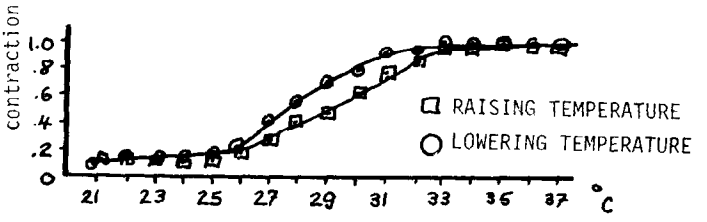

Fig. 3. Effect of changing temperature upon the contraction of the guinea pig ileum irreversibly blocked at 21°.

In another experiment, after 85 per cent of the muscular contraction was blocked at 37° by the inhibitor, the temperature was then lowered to 21°. We observed that essentially no response was elicited by acetylcholine. Upon warming the tissue bath to 37°, the response of the smooth muscle returned to its previous degree of blockade. One possible explanation for the apparently greater blockade at the lower temperature despite a smaller fraction of receptors occupied is that a smaller number of "elements" may be involved in the contraction of the muscle at the lower temperature. Hence, occupation of a smaller fraction would be sufficient to block contraction. This explanation is in harmony with the observations of Taylor (8) and Ehrenpreis and Rosen (10).

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