Effect of pH on Twitch Facilitating Potency of 3-Hydroxyphenyltriethylammonium Ion.* (25170)

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Administration of certain quaternary ammonium compounds to indirectly stimulated nerve muscle preparations results in a marked increase in response to single supramaximal stimuli. A recent report(1) presented experimental evidence that the facilitation is the result of an action of drugs on the motor nerve terminal. Under the influence of these compounds the nerve responds to a single stimulation shock with a brief repetitive discharge, which, in turn, causes a brief tetanic contraction of the muscle. The agents employed were a series of phenyltrialkylammonium ions. The twitch facilitation and repetitive discharge in the nerve, however, were produced only by compounds bearing a meta hydroxy group. The presynaptic action was particularly pronounced with 3-hydroxyphenyltriethylammonium ion (3 OH PTEA). This compound has a pKa of 8.03 (Gill, E., personal communication). In biologic solutions, therefore, it exists as a mixture of ionized and unionized phenol. The present study was undertaken to determine the relative importance of 2 species in producing the twitch facilitation. This was done by comparing the twitch facilitating potency of 3 OH PTEA on the rat phrenic nerve diaphragm preparation in media of pH 7.0, 7.5, and 8.0. Since the proportion of molecules with an ionized hydroxyl hydrogen increases with increasing pH it was anticipated that changes in the apparent potency with pH would reveal whether the ionized form or the unionized form was the more potent in producing potentiation. To obtain an estimate of amount of change which might occur under these extremes of pH, independent of the presence of 3 OH PTEA, a comparative study was done employing a compound which is not affected by pH, tetraethylammonium ion (TEA). Although the mechanism of action of this compound has not been investigated it is also known to increase twitch height in nerve-diaphragm preparations(2).

Method. Diaphragms obtained from 125 g male rats of the Long-Evans strain were employed in a procedure similar to that described by Bulbring(3). The bathing medium contained, in mmols/L, Na^+, 142; K^+, 5.9; Mg^{++}, 1.2; Ca^{++}, 2.5; Cl^-, 127; HCO^-, 25; SO_4^{--}, 1.2; PO_3^{---}, 1.2 and glucose 200 mg%. It was constantly bubbled with 95% O_2 and 5% CO_2. Under these conditions this solution had a pH of 7.5. Solutions of pH 7 and 8 were obtained by modifying the amount of $NaHCO_3$ used in preparing the medium. Temperature was maintained at 37°C by a water jacket about the chamber. Supramaximal stimuli in the form of square waves of 0.1 msec duration were delivered to the nerve from a Grass Stimulator at the rate of 12/minute. Twitches were recorded on a kymograph by a light isotonic level. After a control period had been recorded the drug, dissolved in 0.1 to 1 ml of distilled water, was rapidly added to the 100 ml of medium contained in the bath. The actions of both agents were fully reversible upon washing the preparation with fresh Krebs' solution. Each diaphragm, therefore, was used to test several concentrations of a drug at each pH level. The sequence in which the combinations of concentrations and pH were tested was determined at random prior to each experiment.

Results. The results are presented in Figs. 1 and 2. The 2 agents, 3 OH TEPA and TEA, had a similar, dual action on the nerve diaphragm preparation. Twitch height was increased in presence of low concentrations of the material, but high concentrations produced a blockade of neuromuscular transmission. Intermediate concentrations produced a biphasic response. Initially the twitch was increased in height, but in time this was superseded by a blockade. In the Figures, maximal

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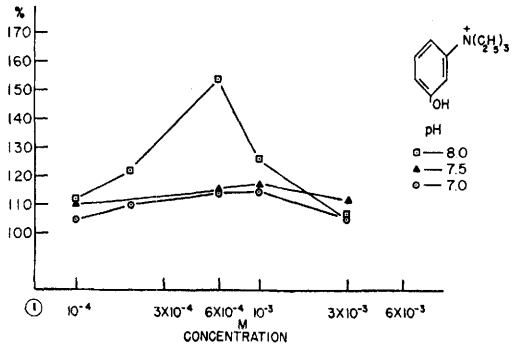

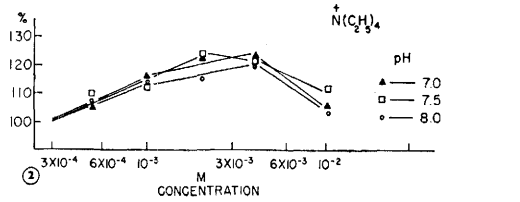

FIG. 1. Effect of pH on concentration response curve for 3-hydroxyphenyltriethylammonium ion. Response is height of twitch of rat phrenic nerve diagram preparation after addition of drug, expressed as percent of control.

FIG. 2. Effect of pH on concentration response curve for tetraethylammonium ion. Response is height of twitch of rat phrenic nerve diaphragm preparation after addition of the drug, expressed as percent of control.

twitch height obtained, expressed as percent of control, is plotted on the ordinate and concentration of drug in the medium is plotted on a logarithmic scale on the abscissa. Each point illustrated represents the mean of 4 to 6 observations.

Over the concentration ranges illustrated both compounds produced an increase in twitch response. At each point the response was significantly greater than control ($p < 0.05$). Concentrations lower than those illustrated produced no significant effect and higher ones rapidly produced a blockade without significant preceding facilitation. At pH 7.0 and 7.5 the increase due to presence of the 3 OH TEPA (Fig. 1) was small and of approximately the same magnitude as that produced by TEA (Fig. 2). At pH 8, however, the potentiation produced by 3 OH TEPA, in the concentration range of 2×10^{-4} to 1×10^{-3}, was significantly greater ($p < 0.05$) than that observed at pH 7.0 and pH 7.5. A similar pH effect did not exist for TEA.

Discussion. It is apparent, therefore, that although presence of a hydroxyl group in a compound is not a pre-requisite to twitch facilitation in this preparation, the ionized form of the phenol is considerably more potent in this regard than is the unionized form. Consequently, the hydroxyl group cannot be contributing to the action by forming a hydrogen bond between the phenol hydrogen and groups in the receptor. Any binding which may occur between the hydroxyl group and the receptor must either be effected by means of electron pairs of oxygen or be an electrostatic attraction between the compound and the receptor. In this regard the binding to presynaptic receptors appears to be quite different from that described by Wilson and Quan (4) for the reaction between close analogs of 3 OH PTEA and the acetylcholinesterase enzyme. On the basis of studies of the pH dependence of the reaction of 3-hydroxyphenylethyldimethylammonium(5) and 3-hydroxyphenyltrimethylammonium(4) ions with the enzyme they concluded that the unionized phenolic hydroxy group contributed more to the binding than the ionized form did and that this probably occurred through the formation of a hydrogen bond.

Summary. Ability of 3-hydroxyphenyltriethylammonium ion to facilitate the response of rat phrenic nerve diaphragm to single supramaximal stimuli has been tested for pH dependence. It is concluded that the phenolate ion is more active in this regard than is the unionized phenol, and that, therefore, the receptor sites for this action on the motor nerve terminals differ from those ascribed to the cholinesterase enzyme.

1. Riker, W. F., Roberts, J., Standaert, F. G., Fujimori, H., *J. Pharmacol. Exp. Therap.*, 1957, v121, 286.

2. Kensler, C. J., *Brit. J. Pharmacol.*, 1950, v5, 204.

3. Bulbring, E., *ibid.*, 1946, v1, 38.

4. Wilson, I. B., Quan, C., *Arch. Biochem. Biophys.*, 1958, v73, 131.

5. ———, *Arch. Intern. Pharmacodynamie*, 1955, v104, 204.

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