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THE EFFECTS OF pH ON THE ACTIVITY OF CORYNEINE AND RELATED PHENOLIC QUATERNARY AMMONIUM SALTS ON THE FROG RECTUS PREPARATION

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1 The activity of *m*-hydroxybenzyltrimethylammonium, coryneine (3:4-dihydroxyphenethyltrimethylammonium, 'quaternary dopamine'), and *m*-hydroxyphenylpropyltrimethylammonium relative to tetramethylammonium has been measured on the frog rectus preparation (*Rana pipiens*) at pH 7 and pH 9.

2 The compounds are more active in the more acid environment indicating that ionization of the phenolic group reduces activity to between one-half and one-tenth of that of the form with the intact hydroxyl group.

3 In contrast with the situation at aminoacid receptors, there is no reason to believe that at other receptors zwitterions are likely to be more active than the uncharged forms with which they are in equilibrium.

Introduction

Because the pK_a values of many biologically interesting phenolic and catecholic amines indicate that there is significant ionization of their hydroxyl groups at physiological pH (Armstrong & Barlow, 1976), it is desirable to know whether it is the phenate or phenolic form which is the more active. This paper describes an attempt to investigate the problem by observing the effects of changing pH on the activity of some phenolic quaternary ammonium salts on the frog isolated rectus preparation, relative to the activity of tetramethylammonium.

Responses can be obtained with the frog rectus muscle over a considerable range of pH. Kalow (1954) obtained results with pH values between 6.7 and 8.7 and Hamilton (1963) with values between 7.04 and 8.86. With phenolic quaternary ammonium salts only the ionization of the phenolic group will be affected by changes in pH and with two of the compounds tested, *m*-hydroxybenzyltrimethylammonium and coryneine (3:4-dihydroxyphenethyltrimethylammonium; the quaternary derivative of dopamine), the $p\bar{K}_a$ at 25°C was the same, 8.75, and such that a change from pH 9 to pH 7 should alter the ratio of phenate to phenolic hydroxyl from about 2:1 to about 1:50 in the more acid environment. If the ionized phenate is the more active, then the activity relative to a substance such as tetramethylammonium, whose ionization will be unaffected by changes in pH, should be very much less at pH 7 than at pH 9. The third substance tested, *m*-hydroxyphenylpropyltrimethylammonium, had a pK_a of 9.51 at 25°C in the conditions described by Armstrong & Barlow (1976; the values are for 10 mM solutions and are not independent of concentration even though corrections for activity coefficients have been applied).

Methods

The rectus abdominis muscle from *Rana pipiens* was set up as described by Edinburgh Staff (1974) at room temperature (16–18°C) and with the contractions recorded isotonically. The two preparations obtained from each frog were set up, one at pH 7 and the other at pH 9, in aerated frog-Ringer solution containing (mM): NaCl 115, KCl 2, $CaCl_2$ 1.8, tris-(hydroxymethyl)aminomethane 10, glucose 11 and HCl either 9 mM or 0.8 mM (the exact concentration depended on any adjustments necessary to obtain the desired pH, which was measured with a glass electrode and estimated to be correct to ± 0.05 pH units).

Drug solutions were made up in a 10 ml stoppered measure, poured onto the preparation and allowed to act for 5 min, and there was an interval of 16 min between doses. In each comparison two concentrations of test drug and of tetramethylammonium were used which produced large and small responses and these were given in such a way that alternate large and small responses were obtained. Usually each con-

centration of each compound was tested only twice in all; possible advantages to be expected from testing them more often seemed to be outweighed by likely changes in the sensitivity of the preparation during the additional time required (it took over 2 h to complete a single comparison as described above). It seemed preferable to compare the activity relative to tetramethylammonium on pairs of muscles, each kept at a particular pH, rather than to attempt to compare the relative activity on the same preparation with the pH altered, because Hamilton (1963) had found that the result was affected by the order in which the alteration was made (i.e. whether the change was from the more acid pH to the more alkaline or *vice versa*).

The samples of *m*-hydroxybenzyltrimethylammonium iodide, coryneine (3:4-dihydroxyphenethyltrimethylammonium iodide), and *m*-hydroxyphenylpropyltrimethylammonium iodide were those used by Armstrong & Barlow (1976). Fresh dilutions of coryneine were made for each experiment because dilute solutions decomposed appreciably in 24 hours.

Results

The results are summarized in Table 1, which shows the mean estimates at the two pH values of the equipotent molar ratios for the compounds relative to tetramethylammonium (i.e. the number of molecules of the compound needed to produce the same effect as one molecule of tetramethylammonium). The ratio of these means gives an estimate of the effect of pH on activity. Alternatively a ratio can be calculated for the results obtained at the two pHs with each pair of preparations and the mean ratio is included in the table with the standard error and number of tests. The mean ratio and the ratio of the means are in close agreement.

It is clear that the phenolic form is more active than the phenate but the latter is not completely inactive. If the ratio of the activity at pH 7 to that at pH 9 is R, the proportion of unionized hydroxyl is U_7 at pH 7 and U_9 at pH 9, and if the activity of the ionized phenate relative to the unionized hydroxyl form is X,

$$R = \frac{U_7 + (1 - U_7)X}{U_9 + (1 - U_9)X}$$

$$\text{and } X = \frac{U_7 - RU_9}{(R - 1 + U_7 - RU_9)}$$

Values of U_7 and U_9 calculated from the dissociation constants determined at low ionic strength and at 25°C, and the corresponding values of X are given in Table 1. At the temperature of the assays and the ionic strength of the frog-Ringer the pK_a values of all the compounds tested will be greater than those determined by potentiometric titration, perhaps by as much as 0.2 units (Armstrong & Barlow, 1976). The

Table 1 Mean estimates at two pH values of equipotent molar ratios (e.p.m.r.) of compounds used, relative to tetramethylammonium

Compound	*Mean e.p.m.r.*		*Mean ratio*
	pH 7	*pH 9*	
m-Hydroxybenzyltrimethylammonium	1.78 ± 0.04 (5)	2.67 ± 0.11 (5)	1.51 ± 0.10 (5)
Ratio of means	1.50		
Fraction unionized	0.982 (0.989)	0.36 (0.47)	
Ratio (X) of activity of phenate to phenolic:	0.47 (0.36)		
Coryneine	0.0380 ± 0.0030 (6)	0.0722 ± 0.0048 (6)	1.95 ± 0.18 (6)
Ratio of means	1.89		
Fraction unionized	0.982 (0.989)	0.36 (0.47)	
Ratio (X) of activity of phenate to phenolic:	0.25 (0.10)		
m-Hydroxyphenylpropyltrimethylammonium	0.00757 ± 0.00092 (5)	0.00870 ± 0.00068 (5)	1.19 ± 0.12 (5)
Ratio of means	1.15		
Fraction unionized	0.997 (0.998)	0.76 (0.84)	
Ratio (X) of activity of phenate to phenolic:	0.45 (0.18)		

The equipotent molar ratios (e.p.m.r.) are relative to tetramethylammonium and the results confirm the very high activity of *m*-hydroxyphenylpropyltrimethylammonium observed by Barlow, Thompson & Scott (1969). The mean ratio is the mean of the ratios of the activity (relative to tetramethylammonium) at pH 7 to that at pH 9 for each pair of preparations; the ratio of the means is calculated from the mean values of e.p.m.r. at the two pHs. The fraction unionized refers to the phenolic group, the quaternary trimethylammonium group remains fully ionized. The values for the fraction unionized and the ratio (X) shown in parentheses have been calculated from values of pK which are bigger by 0.2 units from those obtained by potentiometric titration.

effect of this increase in the pK_a values is that the change in ionization produced by the change in pH is reduced and X, the ratio of the activities of the unionized and ionized forms, is increased. The recalculated values are shown in parentheses in the table. Because of the errors attached to the values of R as well as to U_7 and U_9, it is difficult to obtain a precise estimate of the ratio, X, of the activities of the two forms, particularly for *m*-hydroxyphenylpropyltrimethylammonium where the effect of the pH change on ionization is small and R is close to unity. However, it seems that the presence of the negative charge lowers the activity to between one-half to one-tenth of that of the phenolic compound and has a bigger lowering effect on coryneine (with two phenolic groups) than on the other two compounds.

Discussion

Although these results only apply to phenolic quaternary ammonium salts acting at nicotine-sensitive receptors, they indicate differences from the other common class of zwitterions, amino acids. Zwitterion constants for amino acids are high (see, for instance, Albert & Serjeant, 1962) and the charged nature of the species is considered to be important for actions at amino acid receptors. It is therefore extremely interesting that for highly active phenolic compounds at nicotine-sensitive receptors the activity is associated with the free hydroxyl group rather than charged phenate. The same is true for blocking activity at these receptors by (+)-tubocurarine (Kalow, 1954) and seems likely to be true for actions of phenolic and catecholic amines at adrenoceptors (Armstrong & Barlow, 1976).

In these experiments it was noted that bigger concentrations of tetramethylammonium were needed to produce responses in the more acid environment. This could indicate suppression of ionization of negatively charged groups within the receptor with which the quaternary nitrogen atoms of the compounds interact but this is not the only possible explanation.

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