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THE IMPORTANCE OF IONIZATION IN THE ACTIVITY OF SYMPATHOMIMETIC AMINES

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Several examples exist of the importance of ionization to the biological activity of a compound. Albert and his co-workers (Albert, Rubbo, Goldacre, Davey, and Stone, 1945; Albert and Goldacre, 1948) have shown that ionization is one of the limiting factors in the antibacterial activity of the acridines. Bell and Roblin (1942) showed that in the sulphonamide series the presence of both anions and neutral molecules is necessary for activity. Other workers have shown that ionization is disadvantageous to the narcotic activity of the barbiturates (Clowes, Kletch, and Krahl, 1940). Thus it has been established that ionization is an important factor in the activity of synthetic drugs.

The present investigation was designed to investigate the importance of ionization for the activity of naturally occurring physiological agents. The sympathomimetic amines were chosen as a suitable representative series. It was proposed to examine a group of these amines in order to ascertain which particular ionic species was prevalent and also to examine their pharmacological potency at various *p*H values, in order to discover a relation, if any, between pharmacological activity and change of *p*H, i.e. degree of ionization.

Thus it could be ascertained if ionization is an important factor in determining the activity of sympathomimetic amines; which ionic species is responsible for their activity; and whether small changes in *p*H (as might be found in various tissues) would affect their activity either quantitatively or qualitatively.

METHODS

Determination of Dissociation Constants

Potentiometric Method.—In the potentiometric titrations a Stadie electrode system having a glass electrode was used with a Cambridge *p*H meter, and readings were made at 20° C. Pure nitrogen was passed through the apparatus for about 1 hr. before use. The amines were prepared in 0.01M solution and 0.1N-NaOH was added by means of a 2 ml. microburette. To avoid oxidation, particularly of the diphenolic amines, at high *p*H values, the determinations were carried out anaerobically, and all solutions were made up in boiled, glass-distilled water which had been bubbled with nitrogen. The use of 0.1N-NaOH avoided a too-large alteration of volume during titration. A constant ionic strength (0.1) was employed to avoid salt effects, although slight changes occurred during the titrations.

Most of these compounds have two groups which ionize in the *p*H range examined (amino and phenolic), and a third group (secondary phenolic) which ionizes at very high *p*H values. This latter group in catechol was reported by Abichandani and Jatkar (1938) to have a *p*Ka of over 12.0. Thus, the ionization of the primary phenolic group in the diphenols was assumed not to be affected by the presence of the second phenolic hydroxyl.

The *p*Ka's (i.e. negative logarithms of the dissociation constant Ka) were calculated according to the following equation:

$$K_1 = \frac{h\{(c-a) + \bar{oh}\}}{a - \bar{oh}}$$

where K_1 = dissociation constant, h = hydrogen ion concentration, $\bar{oh}$ = hydroxyl ion concentration, a = equivalents of alkali, c = original concentration of base.

Where it was found that the amino and phenolic groups ionize in close proximity to one another on the *p*Ka scale, then in such cases, in order to calculate the *p*Ka values, it was necessary to use the equation of Britton (1942):

$$\frac{a + h - \bar{oh}}{h + 2K_2} = \frac{cK_1}{h^2 + hK_1 + K_1K_2}$$

where K_2 is the second dissociation constant. The *p*H was recorded and the *p*Ka calculated after each of 7 or 8 additions of alkali. Each addition of alkali resulted in approximately 10% neutralization.

Spectrophotometric Method.—The method involved measurement of the optical density of 0.0002M solutions of the amines (adjusted to ionic strength 0.1) at various *p*H's over wavelengths from 260 to 300 mμ. Glycine buffers were used and the optical densities were measured with a Unicam U.V. spectrophotometer. The procedure was carried out in the absence of oxygen. Fig. 1 shows

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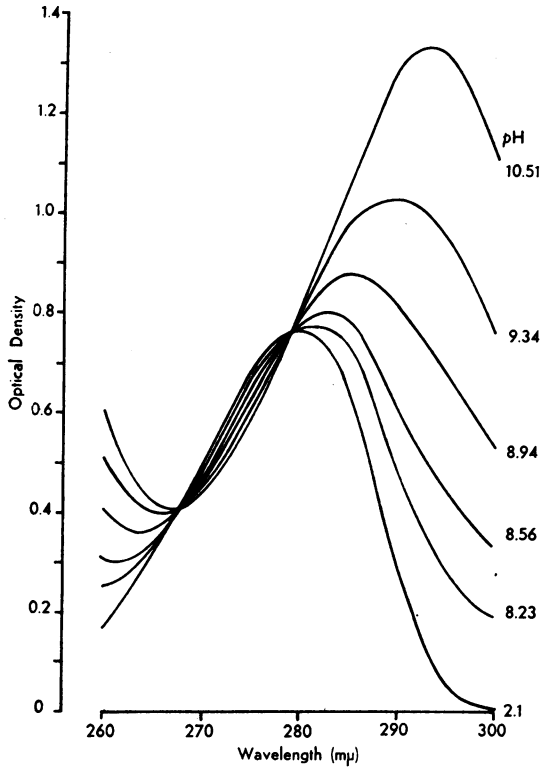


FIG. 1.—To show the change in the ultraviolet absorption spectrum of adrenaline when ionization takes place. This change is shown from unionized molecules at *p*H 2.1 to complete ionization at *p*H 10.51, through four intermediate stages. The concentration of solution was 0.0002M and the ionic concentration 0.1.

typical effects of *p*H on the absorption spectrum of a phenolic amine. The *p*Ka's were obtained from the equation

$$p\mathrm{Ka} = p\mathrm{H} - \log. \left\{ \frac{(\varepsilon - \varepsilon_{AH})}{(\varepsilon_{A^-} - \varepsilon)} \right\}$$

where ε_{AH} and ε_{A^-} are the extinction coefficients of unionized and completely ionized species calculated from the optical densities measured at *p*H 2.1 (ε_{AH}) and *p*H 10.51 (or *p*H 12.48) (ε_{A^-}), ε is the extinction coefficient of the sample at various intermediate *p*H's. Each experiment was carried out and the *p*Ka's calculated at several wavelengths. It was assumed that in this series the presence of the amino group does not affect the absorption spectrum of the phenols.

Pharmacological Methods

The sympathomimetic amines exhibit two types of activity on smooth muscle—relaxation and contraction—according to the preparation employed. In this investigation two preparations were used—isolated rabbit uterus, which is contracted, and isolated rabbit intestine, which is relaxed. Both these tissues were used in a standard organ bath surrounded by a suitable saline (Locke's solution for uterus and Tyrode solution for intestine) maintained at *p*H 7.3 and 37° C., and continuously bubbled with 5% CO_2 in O_2. Each of the amines was assayed against (−)-adrenaline as a standard, and the potency was expressed in terms of adrenaline as 100.

In some experiments the *p*H of the surrounding solution was altered by addition of glycine or glycylglycine buffers. In these experiments the change in the ratio adrenaline activity : amine activity was noted, thus eliminating any tissue reaction to the buffer.

RESULTS

The dissociation constants were determined, where possible, by both methods. It is not possible, using potentiometric titration, to determine which group is ionizing first. Thus, use was made of the spectrophotometer to determine the dissociation of the phenol, as this group has an U.V. absorption spectrum which changes when ionization takes place. There was general agreement between the values obtained by the two methods, those values obtained by the spectrophotometric method being usually 0.1–0.2 of a unit higher. However, with those compounds in which the *p*Ka's of the two ionizing groups were within 0.5 of a unit, there were discrepancies of the order of 0.5. Such compounds were those monophenols containing an alcoholic group in the side chain, e.g. WIN 5512, *p*-sympatol, WIN 833, WIN 5513, and *m*-sympatol. It is considered that when the two *p*Ka's are so close the potentiometric method used here is not sufficiently accurate for direct application of Britton's equation. In these instances the phenolic $p\mathrm{Ka}_1$ was determined by the spectrophotometric method and the basic $p\mathrm{Ka}_2$ calculated from this result and Britton's equation.

The values of the dissociation constants expressed in *p*Ka units are given in Table I. The basic *p*Ka's are greater than 9.4 and the acidic *p*Ka's are, with one exception, over 8.8. Thus, at physiological *p*H, the ionic constitution of these amines is approximately 94% kation, 5% zwitterion, and 1% anion and undissociated molecule. Leffler, Spencer, and Burger (1951) measured the apparent dissociation constants of 27 sympathomimetic amines. Their method was to add to a solution of the salt of each amine the calculated amount of sodium hydroxide solution required for half-neutralization—a procedure which is liable to give rise to undetectable errors. Since the phenolic group dissociates first, these authors were measuring its dissociation in the diphenols, that of the basic group in the non-phenols, and a complex dissociation of the two groups in the monophenols. These results have not been taken into account during the present investigation. The

TABLE I

DISSOCIATION CONSTANTS (± STANDARD ERRORS) OF THE PHENOLIC (pK_1) AND AMINO (pK_2) GROUP OF SYMPATHOMINETIC AMINES

The numbers represent the potency of each amine compared with (−)-adrenaline as 100. In the last column are given the rates of oxidation by amine oxidase relative to that of tyramine as 100.

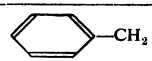
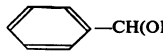
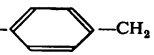
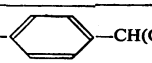
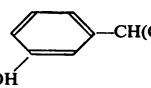
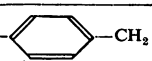
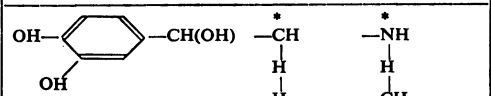
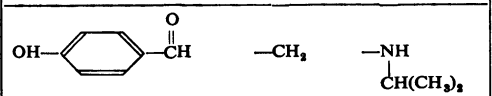
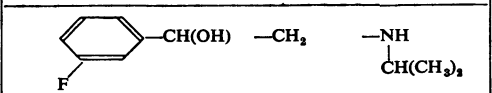
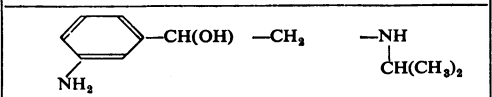
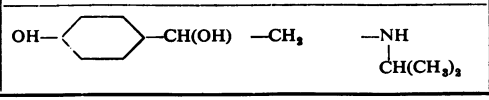
Compound	Substituents on Atoms Indicated by *	pKa_1		pKa_2	Pharmacol. Activity		Enzymatic Oxidation (Blaschko *et al.*, 1937)
		Spectro.	Potentiom.	Potentiom.	Rabbit Intestine	Rabbit Uterus	
Phenylethylamine							
β-Phenylethylamine				9·86±0·06	<0·01	<0·01	11
(±)-Phenylisopropylamine				9·93±0·01	<0·01	0·02	<2
WIN 5523				10·02±0·06	0·04	<0·01	—
Phenylethanolamine							
(−)-Ephedrine				9·49±0·05	0·05	0·06	<2
(−)-Propadrine				9·44±0·04	0·01	0·13	—
Mono OH-Phenylethylamine							
Tyramine		9·77±0·06	9·53±0·03	10·78±0·02	<0·01[1]	0·05	100
WIN 5565		9·70±0·04	9·49±0·02	10·75±0·04	0·02	<0·01	—
Mono OH-Phenylethanolamine							
WIN 5512		9·53±0·07		9·70±0·09	0·5	0·05	—
(±)-*p*-Sympatol		9·59±0·05		9·71±0·08	0·5	1·0	48
WIN 833		9·57±0·07		9·78±0·09	1·0	<0·01	—
WIN 5513		9·56±0·08		9·76±0·09	<0·01[1]	<0·01	—
(−)-*m*-Sympatol		9·61±0·09		9·70±0·07	10·0	60·0	59
Di OH-Phenylethylamine							
Hydroxytyramine		8·92±0·06	8·87±0·03	10·63±0·07	0·2	0·2	—
Epinine		8·86±0·08	8·90±0·06	10·61±0·06	8·0	10·0	125

TABLE I—*contd.*

Compound	Substituents on Atoms Indicated by *	pKa_1 Spectro.	pKa_1 Potentiom.	pKa_2 Potentiom.	Pharmacol. Activity: Rabbit Intestine	Pharmacol. Activity: Rabbit Uterus	Enzymatic Oxidation (Blaschko *et al.*, 1937)
Di OH-Phenylethanolamine							
(−)-Noradrenaline		8·90±0·06	8·73±0·04	9·78±0·09	100	90	51
(−)-Adrenaline		8·88±0·04	8·71±0·06	9·90±0·06	100	100	65
(−)-Isopropylnoradrenaline		8·87±0·07	8·72±0·05	9·87±0·07	130	<0·01	—
WIN 5589		8·89±0·06	8·85±0·04	10·03±0·06	100	0·01	—
(−)-Corbasil		8·85±0·05	8·75±0·03	9·75±0·09	12	3·3	<2
WIN 3243		8·91±0·08	8·85±0·05	10·00±0·06	5	<0·01	—
Miscellaneous							
WIN 5514		7·64±0·03	7·53±0·02	9·58±0·09	<0·01	<0·01	—
WIN 5505				9·48±0·05	0·5	<0·01	—
WIN 5503			4·22±0·04[4]	9·60±0·04	0·01	<0·01	—
WIN 5579[3]				10·23±0·07	<0·01	<0·01	—

[1] Caused non-sympathomimetic contraction. [2] Amino group $-N(CH_3)_2$. [3] Phenyl replaced by cyclohexyl. [4] Basic pKa of N atom attached to phenyl ring. — Indicates not tested.

dissociation constants of five of the compounds have been reported by others workers: *laevo*ephedrine 9.48 (Ayrapaa, 1950); β-phenylethylamine 9.85 (Bardinet and Metayer, 1948); tyramine (NH_2) 9.3, (OH) 10.9 (Ogston, 1936); adrenaline (NH_2) 10.2, (OH) 8.7; tyramine (NH_2) 9.3, (OH) 10.9 (Albert, 1952). The first two of these results agree with those found in the present investigation, and those for adrenaline differ only by 0.2–0.3. The results for tyramine, however, are quite different, and it is obvious that the figure given by Ogston (1936) and Albert (1952) for the ionization of the phenolic group has been found in the present investigation to represent that of the amino group and *vice versa*.

Some generalizations may be made on the relationship between chemical structure and dissociation constants in this series of phenylethylamine derivatives. This constant of the basic amino group is reduced by 0.4 of a *p*Ka unit on addition of the alcoholic group when no phenol group is present, and by 0.8 of a unit in mono- and di-phenols. The phenolic phenylethylamines are considerably more basic (0.8 unit) than their non-phenolic analogues. The acidic phenol group is more feebly ionized in the monophenols than in the diphenols. It is interesting to note that, when the alcoholic group in the side chain is replaced by a ketonic group in a *para*-monophenol, the acid is greatly strengthened and the base weakened, e.g. WIN 833, acid 9.59, base 9.78; WIN 5514, acid 7.64, base 9.58. In the cyclohexylethylamine derivative (WIN 5579) the basic group is stronger than that of the corresponding phenylethylamine (WIN 833), while the hydroxyl loses its phenolic character and does not ionize at all.

Table I also shows the relative pharmacological activities of the compounds in relaxing rabbit intestine and in contracting rabbit uterus. It is quite obvious that there is no direct relationship between pharmacological activity and *p*Ka. The only notable feature is that the compounds are present mainly in the kationic form at physiological *p*H. The *p*K's of these compounds are such that to ensure the absence (effectively) of the kationic form, the *p*H would have to be raised to 9.5–10.0, which is unsuitable for a biological tissue. In a series of experiments in which the *p*H of the medium surrounding the biological test tissue was varied, it was found that glycine and glycylglycine buffers (Dernby, 1915) provided a suitable means of raising the *p*H to 8.5–8.7 without any apparent effect on the tissue, but at higher *p*H's irreversible changes took place. There was no change in the relative activities of the compounds over this range of *p*H.

In the last column of Table I are given some figures quoted from Blaschko, Richter, and Schlossman (1937) of relative rates of oxidation by the enzyme amine oxidase. There is no relationship between enzymatic oxidation and *p*Ka, and therefore no evidence regarding the preference of this enzyme for any particular ionic species.

Discussion

The results of this investigation give a general picture of the ionization of the sympathomimetic amines derived from phenylethylamine. The alcoholic group in the side chain has a base-weakening effect whatever other structures are present, while the phenolic hydroxyl strengthens the base. In the presence of a second phenolic group which ionizes only at very high *p*H's, the dissociation of the ionizing phenol is enhanced. Other structural modifications have been made, but these did not result in a considerable weakening of the base. Thus, it seems unlikely from these facts that a sympathomimetic agent based on the structure of phenylethylamine can be derived having a basic *p*Ka of less than 8.0; above this *p*Ka no appreciable change in ionic composition results from small changes in physiological *p*H.

It is concluded that the main question—how important ionization is for the activity of sympathomimetic amines—cannot be answered. It can be stated, however, that ionization cannot account for the differences in activity among sympathomimetic amines derived from phenylethylamine. Further, at physiological *p*H the kationic form of these compounds is by far in excess of any other ionic species and is thus probably responsible for pharmacological activity, but other possibilities cannot be excluded.

Summary

1. A series of sympathomimetic amines has been examined in order to discover the importance of ionization for their pharmacological activity.

2. These amines are strong bases of *p*Ka (amino) 9.4–10.8, and weak acids of *p*Ka (phenolic) 8.8–9.5.

3. Ionization is not important in accounting for the differences in activity of these amines.

4. At physiological *p*H the compounds are present almost wholly as kations.

It is a pleasure to acknowledge much helpful discussion with Dr. A. Neuberger of this Institute.

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