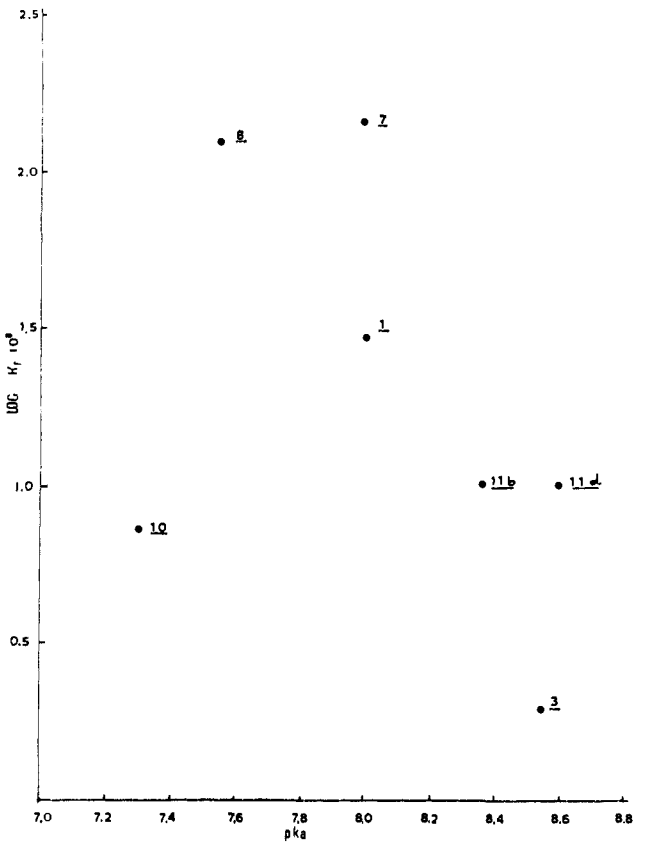


Figure 7.—Relationship between $k_r$ and p$K_a$ for a number of reactivators of DFP-inhibited AChE.

reaction on the enzyme (the lower the value of $K$, the higher the affinity to the enzyme).[16] No distinction is made here between oximate anion or its conjugate acid. The first-order rate constant, $k_r$, on the other hand, is independent of affinity and, therefore, should be a function of the basicity or nucleophilicity of the reactivator molecule in a series, provided steric parameters are kept equal for all members of the series. A plot of log $k_r$ against p$K_a$ values for the oximes **1**, **3**, **7**, **8**, **10**, and **11** reveals that the most reactive members are not necessarily the most nucleophilic ones, but rather those having a p$K_a$ value in the range 7.6–8.0 (Figure 7). It must be noted that for the symmetrical oximes **7** and **8**, only the first p$K_a$'s ought be considered since it is highly unlikely that both oximate groups on a single molecule have simultaneous access to the phosphoryl residue in the enzyme active site. As for the oxime **11**, the p$K_a$ values of both forms b and d are given, although it is believed that there should be a preponderance of the former form at pH 7.4 [macroscopic p$K_a$ of the amino group = 8.82[1]] either because of extensive protonation or donation of the nitrogen lone pair of electrons through another process.

In this context, application of the equation of Epstein, *et al.*,[17] relating reactivity, p$K_a$, and pH should be revealing. $K_a = [H^+](1 - \alpha/\alpha)$, if $[H^+] = 4.10^{-8}$ and $\alpha = 0.619$ ($\pm 0.105$) from eq 5, then $K_a = 2.46 \times 10^{-8}$ or p$K_a$ 7.6.

In other words, a member of a series possessing a p$K_a$ of 7.6 should also exhibit the highest $k_r$ value at pH 7.4. At this state of knowledge, these conditions are in fair agreement with the experimental results.

(16) For an excellent treatment of the case of chymotrypsin, see B. F. Erlanger, "Proceedings of the Conference on Structure and Reactions of DFP Sensitive Enzymes," E. Heilbronn, Ed., Swedish Research Institute of National Defence, Stockholm, 1967, p 143.

(17) J. Epstein, H. O. Michel, and W. A. Mosher, *J. Theor. Biol.*, **19**, 320 (1968).

# *Notes*

## Comparative Pharmacology of 5-Hydroxytryptamine and Its Benzofuran, Benzo[*b*]thiophen, and Indene Isosteres

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The proposal by Vane[1] that 5-hydroxytryptamine (5-HT) attaches to its receptor site by a 2-point interaction involving the 5-OH group and the terminal $NH_2$ has received indirect support from experiments with tryptamine isosteres. It has been concluded that replacement of the indolic imino moiety of tryptamines by $CH_2$ or by S does not alter appreciably either the contractile activity of tryptamines upon the rat stomach fundus preparation[2,3] or their central activity in rabbits as measured by quantitative electroencephalography,[4] and it is apparent that the indolic imino group does not participate significantly in interactions with peripheral or central tryptamine receptors. Nevertheless, it is equally apparent that there exist distinct 5-HT and tryptamine receptors;[3,5] for example, phenoxybenzamine blocks the response of the rat stomach fundus to 5-HT, but is much less effective against tryptamines and their isosteres, which can presumably occupy the phenoxybenzamine-resistant tryptamine (PRT) re-

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(2) J. C. Winter, P. K. Gessner, and D. D. Godse, *J. Med. Chem.*, **10**, 856 (1967).

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(4) E. Campaigne, E. S. Neiss, C. C. Pfeiffer, and R. A. Beck, *J. Med. Chem.*, **11**, 1049 (1968).

(5) For reviews, see (a) S. Garattini and L. Valzelli, "Serotonin," Elsevier, Amsterdam, 1965; (b) L. B. Kier, "Fundamental Concepts in Drug–Receptor Interactions," J. F. Danielli, J. F. Moran, and D. J. Triggle, Ed., Academic Press, London, 1970, pp 15–45.

ceptors as well as the phenoxybenzamine-sensitive 5-HT receptors.[3] One previous study[6] has included direct qualitative comparison between 5-HT and its benzo[*b*]thiophen isostere, and the present report now deals with the interaction of 5-HT, and its benzofuran (OAS),[7] benzo[*b*]thiophen (SAS),[8] and indene (CAS)[9] isosteres with 5-HT receptors in the rat stomach fundus preparation.

The results in the table show that the 3 isosteres are significantly ($P < 0.05$) less potent than 5-HT in producing contractions of the rat fundus strip. The order of potency, as determined by $pD_2$ values, was 5-HT > CAS > SAS > OAS, and the value of 7.59 for 5-HT is midway between the values of 7.4 and 7.72 quoted by Winter and Gessner.[3] The slopes of the common regression lines bear an inverse relationship to the $pD_2$ values but the significance of this finding has not been investigated. It is evident that substitution of the indolic imino moiety of 5-HT by O, S, or $CH_2$, has little effect on the relative intrinsic activities, but has a profound deleterious effect on the potency of the compounds in producing contractions of the rat stomach fundus. The conclusion that the indolic NH is important for determining the potency of 5-HT-like activity differs from that evinced by Winter and others[2] for the tryptamines, where it is stated that this group is not important. However, their conclusion seems unjustified because the $pD_2$ values which they quote for tryptamine and its benzo[*b*]thiophen and indene isosteres differ markedly, being 5.84, 4.78, and 5.02, respectively.

An attempt was made to assess the specificity of the 5-HT isosteres for 5-HT receptors in the rat stomach fundus, by measuring the degree of blockade produced by phenoxybenzamine. Reference to Table I shows

TABLE I

ACTIVITY OF 5-HT AND ITS ISOSTERES AS AGONISTS AND DEGREE OF BLOCKADE BY PHENOXYBENZAMINE ON THE RAT STOMACH FUNDUS STRIP

| | Agonist activity | | | Blockade by phenoxybenzamine, $1.84 \times 10^{-4}$ *M* for 20 min | |
|---|---|---|---|---|---|
| Compd | Relative intrinsic activity (±SE) | $pD_2$ (±SE) | Slope | Bath concn of agonist, *M* | % block of agonist response (±SE) |
| 5-HT | 1.00 | 7.59 (±0.14) | 1.00 | $3.5 \times 10^{-6}$ | 95.67 (±0.67) |
| OAS | 0.84 (±0.04) | 4.94 (±0.11) | 0.98 | $1.8 \times 10^{-3}$ | 67.66 (±2.4) |
| SAS | 1.08 (±0.09) | 6.13 (±0.16) | 0.73 | $7.8 \times 10^{-4}$ | 70.6 (±4.3) |
| CAS | 0.96 (±0.09) | 6.60 (±0.53) | 0.62 | $7.5 \times 10^{-4}$ | 78 (±5.07) |

that phenoxybenzamine ($1.84 \times 10^{-4}$ *M*) blocks the effect of a dose of 5-HT which normally produces a 95% maximal contraction to the extent of 95.7%, which is in good agreement with the 98.3% quoted by Winter and Gessner.[3] The isosteres of 5-HT, although less specific than the parent compound, nevertheless exhibited a greater degree of specificity than corresponding tryptamines or their isosteres. Our results indicate that the 5-OH function of 5-HT is important for specificity for 5-HT receptors and confirm the suggestion[6] that the indolic NH affects both specificity and potency of 5-HT-like compounds, at least as far as their contractile activity on the rat stomach fundus is concerned.

It was considered of interest to study the possible central activity of the isosteres since replacement of the indolic NH may affect penetration into the CNS. To elucidate this point the effects of the 5-HT isosteres on rabbit rectal temp were studied, a test which has shown good correlation between hyperthermia and central activity.[10] No significant hyperthermia was detected at doses of 5 mg/kg (iv), although 5-hydroxytryptophan produces significant hyperthermia at doses of 25 mg/kg.[11]

## Experimental Section

**Chemistry.**—5-Hydroxytryptamine creatinine sulfate was purchased from Koch-Light Laboratories. 3-(2-Aminoethyl)-5-hydroxybenzofuran·HCl (OAS) was synthesized by known methods.[7] 3-(2-Aminoethyl)-5-hydroxybenzo[*b*]thiophene·HCl (SAS) was obtained by diborane reduction of 5-hydroxy-3-benzo[*b*]thienylacetamide.[12]

**3-(2-Aminoethyl)-5-hydroxyindene·HCl (CAS).**—A soln of $AlCl_3$ (6.7 g, 50 mmoles) in dry $Et_2O$ (100 ml) was added quickly to a suspension of LAH (1.9 g, 50 mmoles) in dry $Et_2O$ (125 ml). After stirring for 5 min, a soln of 5-hydroxyindene-3-acetonitrile[9] (850 mg, 5 mmoles) in dry $Et_2O$ (150 ml) was added dropwise, and the mixt was allowed to stir overnight at room temp. $H_2O$ was added dropwise to decompose excess LAH and the product was extd into 3 *N* $H_2SO_4$ (200 ml). Work-up of the $Et_2O$ layer did not yield any product. The acid layer was cooled in ice, the pH was adjusted to 8 by careful addition of KOH pellets, and the aq soln was extd continuously with $CHCl_3$ for 24 hr. Subsequent extns were carried out at pH values of 9, 10, and 11. The combined $CHCl_3$ exts were dried ($Na_2SO_4$) and evapd, and the residue was converted into its hydrochloride in EtOH–$Et_2O$. Recrystn from MeOH–EtAc–$Et_2O$ gave the hygroscopic hydrochloride, which was stable under $N_2$: yield, 790 mg (74%): mp 172–173.5°; ir and nmr spectra consistent with those reported for the oxalate.[9] *Anal.* ($C_{11}H_{13}NO$·HCl), C, H, N.

**Pharmacology.**—Fundus strips were prepd from rats of either sex (180–220 g), as described by Vane,[13] and mounted in a 5-ml organ bath containing Kreb's soln at 37° gassed with a mixt of 95% $O_2$ and 5% $CO_2$. Contractions of the strips were recorded on a smoked drum using a pendulum auxotonic lever,[14] with a magnification of 6:1 and a resting load of 1 g. The pendulum was designed such that the load on the muscle increased by 0.1 g/cm deflection of the writing point.

Cumulative dose–response curves were obtained and $pD_2$ values were detd by the method of Van Rossum.[15] A cumulative dose–response curve was first obtained with 5-HT and, after washing out, a recovery period of 40 min was allowed before application of the test compound. The intrinsic activity of the latter was calcd by comparing the maximum contraction produced with the maximum contraction produced by 5-HT on the same prepn. Control experiments showed that reproducible dose–response curves could be obtd with 5-HT using a recovery period of 40 min. Only 1 compd together with 5-HT was tested on each prepn, and each compd was tested on 4 prepns. $pD_2$ values and slopes of common regression lines were calcd by the method of Finney.[16]

The degree of block produced by phenoxybenzamine on contractions elicited by the test compds was determined by the method of Winter and Gessner.[3] Two strips were cut from the same fundus and were used in parallel, one strip as a control unexposed to phenoxybenzamine. The dose of test compds used was the dose that produced 95% of the maximal contraction,

(6) E. Campaigne, R. P. Maickel, F. P. Miller, and T. R. Bosin, *Arch. Int. Pharmacodyn.*, **177**, 360 (1969).

(7) G. Hallmann and K. Hägele, *Justus Liebigs Ann. Chem.*, **662**, 147 (1963).

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(9) R. M. Pinder, *J. Chem. Soc. C*, 114 (1970).

(10) R. W. Brimblecombe, *Int. J. Neuropharmacol.*, **6**, 423 (1967).

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(12) We thank Dr. K. Clarke of the University of Hull, England, for a gift of this compound and for unpublished details of its reduction.

(13) J. R. Vane, *Brit. J. Pharmacol.*, **12**, 344 (1957).

(14) W. D. M. Paton, *J. Physiol. (London)*, **137**, 35P (1957).

(15) J. M. Van Rossum, *Arch. Int. Pharmacodyn.*, **143**, 299 (1963).

(16) D. J. Finney, "Statistical Methods in Biological Assay," Chas. Griffin and Co., Ltd., London, 1964, p 67.

as calcd from the common regression line derived from probit analysis of the cumulative dose–response curves. The dose ($1.84 \times 10^{-4}$ *M*) and exposure time (20 min) of phenoxybenzamine were the same as used by Winter and Gessner.[3]

Rabbit rectal temp measurements were made with Cu–constantin thermocouples on old male rabbits as described by Brimblecombe.[10]

## Synthesis and Pharmacology of an Epoxide Derivative of Ethacrynic Acid

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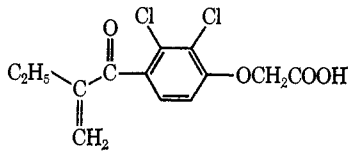
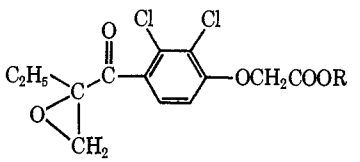
Ethacrynic acid (**1**) is thought to evoke a diuretic response by reacting with protein-bound sulfhydryl groups (PBSH) in renal tubular cells.[2–6] It has been suggested that a Michael-type reaction is involved.[4] In an attempt to explore this hypothesis further, we have synthesized the epoxide derivative **2a** of ethacrynic acid.



**1**



**2a**, R = H
**b**, R = $CH_3$

Unlike ethacrynic acid (**1**) the epoxide **2a** is no longer capable of reacting with SH-containing substances *via* a Michael-type addition; however, it has the potential of reacting with them by an SN2 reaction. The SN2 reaction involving epoxides and SH-containing substances has been postulated to occur both *in vivo* and *in vitro*. Several exogenous epoxides are known to react with glutathione in rabbit[7] or bird[8] liver preparations. In addition, the carcinogenicity of many epoxides is thought to be due to their reaction with various nucleophilic groups (*i.e.*, SH, $NH_2$) of proteins.[9–13]

We reasoned that if a reaction with PBSH in renal tissue is necessary for the diuretic action of ethacrynic acid, then the epoxide **2a** could conceivably alkylate (by an SN2 reaction) these essential groups and act either as a diuretic agent or as an antagonist of the diuretic action of ethacrynic acid.

Ethacrynic acid and its epoxide derivative **2a** were compared on the basis of their diuretic activity, renal $Na^+$- and $K^+$- ATPase inhibitory activity, and *in vitro* reactivity with cysteine. The epoxide **2a** failed to induce a measurable diuretic response in dogs when administered iv in a dose of 2 mg/kg. The method of testing was similar to that used by Small and Cafruny.[14] Even when the iv dose of **2a** was increased to 25 mg/kg, there was no significant diuretic effect. Urine flow was monitored for 30 min after administration of **2a**. The glomerular filtration rate, measured as the renal clearance of inulin, was not affected. When injected directly into tubular lumens by the retrograde intraluminal injection technique[15] the epoxide failed to elicit a diuretic response [3.5 ml of an aq mannitol soln (20% wt/wt) containing 10 mg of the epoxide/ml]. An iv dose of **2a** (2 or 25 mg/kg) 30-min prior to iv administration of ethacrynic acid (2.0 mg/kg) failed to antagonize the normal diuretic response to the latter agent.

Canine renal $Na^+$- and $K^+$- ATPase,[16] an SH-containing enzyme that has been implicated in the renal transport of $Na^+$, was not inhibited by **2a**.[17]

*In vitro* experiments have shown that *ca.* 82% of the epoxide **2a** can be recovered unchanged after a 30-min exposure to an unbuffered aq soln of cysteine adjusted to pH 7.6–7.7. Although the conditions used in this study were slightly different than those employed by Duggan and Noll,[5] it is clear that cysteine reacts with ethacrynic acid at a much faster rate than it does with **2a**. Thus, **2a** differs markedly from ethacrynic acid in all three studies mentioned above.

The relative inactivity of **2a** can be explained in several ways. (1) A Michael-type reaction involving ethacrynic acid and renal PBSH may be required to bring about a diuretic response. The epoxide **2a** cannot participate in a Michael-type reaction and may well be too stable *in vivo* to react with renal PBSH by an SN2 reaction. The *in vitro* stability of **2a** in the presence of cysteine supports the previous statement. (2) It is possible that the Michael-type reaction between ethacrynic acid and renal PBSH occurs but does not contribute to the observed diuresis. If this is the case, it is conceivable that the diuretic response to ethacrynic acid is the result of a specific interaction (other than a Michael-type reaction with PBSH) between the drug and renal receptors that requires an intact $\alpha,\beta$-unsaturated

(1) Abstracted in part from the Ph.D. Dissertation of Daniel A. Koechel, Department of Pharmacology and Therapeutics, Medical College of Ohio, Toledo, Ohio 43614. This study was supported by U. S. Public Health Service Fellowship 5-FI-GM-31,012-02 and U. S. Public Health Service Grant AM 13152.

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(17) The ATPase work was conducted by Drs. Julius Allen and Arnold Schwartz, Department of Pharmacology, Baylor University College of Medicine, Houston, Texas.