Biochemical Pharmacology, Vol. 27, pp. 2015–2019.

0006-2952/78/0815-2015 $02.00/0

INTERACTIONS OF ADRENERGIC COMPOUNDS WITH BRAIN MEMBRANE CONSTITUENTS

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(*Received* 5 *December* 1977; *accepted* 22 *February* 1978)

Abstract—The specific activity of the Na^+, K^+-ATPase of rat brain synaptosomes was modulated by a series of adrenergic compounds in a manner related to each compound's partition between aqueous and organic solvents. The more organic-soluble compounds, the β-2 adrenergic blockers propranolol, pronethalol and butoxamine, inhibited the enzyme between 13 and 30 per cent. The more aqueous-soluble compounds, the agonists, epinephrine, norepinephrine and isoproterenol, and the β-1 blockers, practolol and acebutolol, stimulated the enzymatic activity by 30 per cent. These effects may be due to non-specific membrane interactions rather than to specific receptor effects. Optical measurements with pure protein and phospholipid indicated that the aqueous-soluble compounds bound to protein while the organic-soluble compounds interacted with acidic phospholipid phosphatidyl serine. The possible consequences of the compounds binding with acidic phospholipids and the resulting effect on membrane properties are discussed.

Several β-adrenergic blocking agents have been shown to have a 'non specific' stabilizing or local anesthetic effect on biological membranes [1–3]. The stabilizing effect, originally used to show that local anesthetics protected against hypotonic lysis in erythrocytes, has been observed at concentrations several orders of magnitude higher (10^{-5} to 10^{-3} M) than that necessary for optimum drug-specific receptor interactions [1, 2]. These studies indicated that drugs with greater solubility in non-polar solvents generally had greater anesthetic potency. The adrenergic agents chosen for this study were considered as a group of compounds that had structural homology (Fig. 1), although from a pharmacological point of view, they represented both β-1 and β-2 blockers as well as agonists. The approach was initially to examine the interaction of these compounds with membrane components, at concentrations similar to those used to elicit anesthetic effects. The Na^+, K^+-ATPase, an important intrinsic membrane constituent, was chosen for study since this enzyme requires phospholipid for full activity [4–6] and its activity may reflect modifications to either the protein or lipid region of the membrane. A goal was to determine which membrane components might mediate drug effects on the enzyme and which structural features of the drugs were essential for these effects.

METHODS

Adult male Sprague–Dawley rats were decapitated and the brains removed and rapidly cooled in 0.32 M sucrose, pH 7.4. Synaptosomes were prepared by differential centrifugation, essentially as described by Gray and Whittaker [7]. The resulting material was stored at −80° in sucrose. The Na^+, K^+-ATPase was assayed ± Na^+, using AT[^{32}P] as substrate [8]. For determining the effects of the adrenergic compounds on Na^+, K^+-ATPase activity, the synaptosomes were preincubated in a buffered solution containing the desired concentration of the compound at 37° for 10 min. The subsequent enzyme assay solutions also

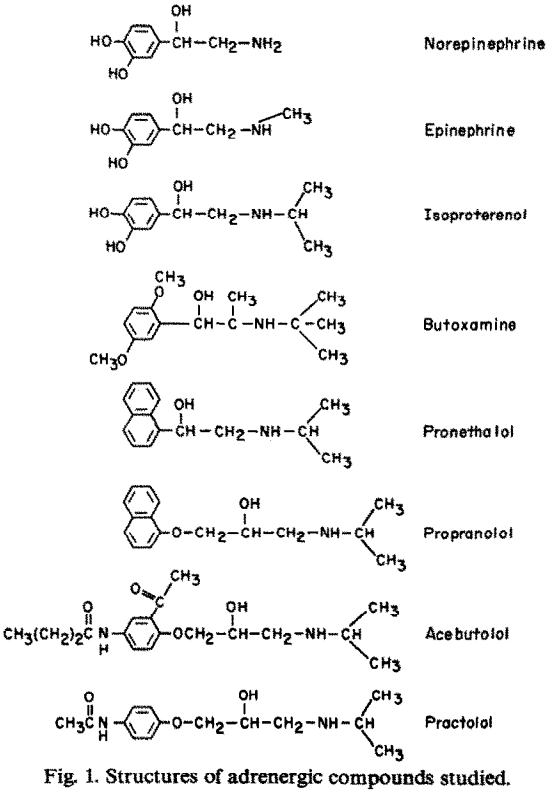

Fig. 1. Structures of adrenergic compounds studied.

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contained an identical concentration of the compound under study. All reported values were obtained from a minimum of three separate determinations, each done in duplicate.

The partitioning of the compounds was performed as described by Wiethold *et al.* [3]. A 0.5 mM drug solution in 0.1 M phosphate buffer, pH 7.0, was mechanically shaken with an equal volume (1 ml) of chloroform for 20 min. The phases were separated by low speed centrifugation and the optical density of the aqueous phase was determined before and after extraction, to determine the concentration of drug in each phase.

The techniques of solvent perturbation difference spectroscopy, as they apply to protein study, have been reviewed by Herskovits [9]. Two double-compartment cuvettes were used. The compartments were in tandem so that initially the compound under study was separated from the protein or phospholipid in each cuvette and a baseline spectrum was run. The compartments of the sample cuvette were mixed to allow interaction between the drug and protein or lipid. About 5 min was allowed for equilibrium before the second spectrum was scanned. The total concentration and optical density were the same in both cuvettes before and after mixing, so the difference between the spectra was due to interaction between the constituents in the sample cuvette. A difference of less than 0.01 O.D. in a solution with a total O.D. of greater than 1 could be easily detected. The adrenergic compounds were used at concentrations of 0.1–0.5 mM in 0.1 M phosphate buffer, pH 7.0; the bovine serum albumin (BSA) concentration was 1.75 mg/ml, and phosphatidyl serine (PS) in phosphate buffer was between 75 and 80 μM, as noted in the legends.

The D and L isomers of propranolol, pronethalol and practolol were products of Ayerst Laboratories, New York, N.Y. and were a gift of Mr. W. D. Northcroft. Acebutolol (Ives Laboratory, New York, N.Y.) and butoxamine (Burroughs Wellcome Co., Research Triangle Park, N.C.) were gifts from Dr. J. Brown. Norepinephrine, epinephrine and isoproterenol were obtained from the Sigma Chemical Co. (St. Louis, MO). Each compound in this collection was made as a stock solution of 50–100 mM in 1 mM HCl. Dilutions with buffer were made prior to the introduction of membrane material.

RESULTS

Effects of drugs on Na^+, K^+*-ATPase activity.* The Na^+,K^+-ATPase within rat brain synaptosomes appeared to be affected by all of the adrenergic compounds examined (Fig. 2). All the β-2 blocking agents, propranolol, pronethalol, and butoxamine, inhibited enzyme activity. The D-optical isomer of propranolol was as effective as the L isomer in producing inhibition and this was taken as evidence that the inhibition was produced by a general membrane phenomenon rather than by a specific interaction at a drug receptor site. In the latter case the L isomer would be 100–500 times more effective than the D isomer [2, 10]. Pronethalol, a close structural homolog of propranolol (Fig. 1), was not as potent an inhibitor as propanolol. Butoxamine, the third β-2 blocker used, was equal to pronethalol in its ability to inhibit the Na^+,K^+-ATPase.

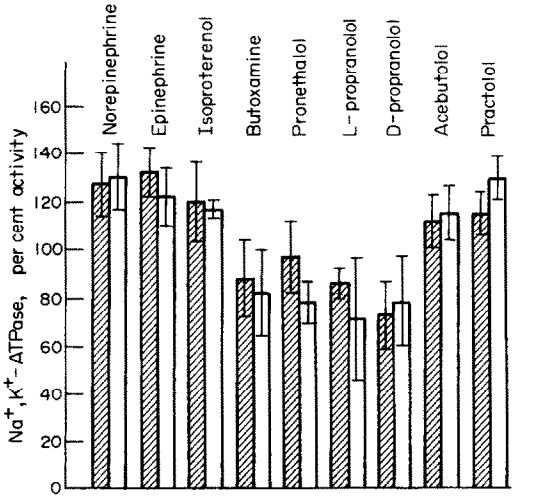

Fig. 2. Effects of adrenergic compounds on the specific activity of the Na^+, K^+-ATPase of synaptosomes. Hatched bars are 250 μM and the open bars, 1 mM, for the compounds examined $\pm$ S.D. for at least three experiments.

The β-1 blockers, acebutolol and practolol, on the other hand caused a stimulation of the Na^+,K^+-ATPase. Practolol (1 mM) caused a 30 per cent stimulation of enzyme activity over controls and was as effective in the stimulation of the agonists used below.

The β-agonists, epinephrine, norepinephrine and isoproterenol, stimulated Na^+,K^+-ATPase activity between 20 and 35 per cent. Stimulations of between 200 and 250 per cent have been reported by others [11–13]. The synaptosome preparation used in this study was isolated from whole rat brain. One previous study used synaptosomes from the hypothalamic region of the brain, which is rich in adrenergic neurons [11] and might be expected to exhibit greater adrenergic sensitivity than whole brain synaptosomes.

In order to correlate the effects of the drugs on enzyme activity with their solubility in the nonpolar phase of the synaptosome membrane, partition coefficients were examined. The partitioning of compounds, with local anesthetic action, between aqueous and organic phases has been correlated previously with membrane-stabilizing capability [1]. The procedure described by Wiethold *et al.* [3] was used to determine the partition coefficients of the adrenergic compounds used in the present study (Table 1). It is evident that all the β-blockers, propranolol, pronethalol, and butoxamine, are more soluble in the organic phase ($CHCl_3$) than in the phosphate buffer. The β-1 blockers, acebutolol and practolol, have solubility characteristics similar to the agonists; they are all more water soluble than the β-2 blockers.

This pattern is similar to the effect each compound exerted on the Na^+, K^+-ATPase (Fig. 2). Table 1 shows that there is a clear correlation between the partition coefficient of each compound and its effect on the Na^+, K^+-ATPase. Those compounds with higher solubilities in the organic phase inhibited activity while the more water-soluble compounds stimulated the enzyme.