Table 1. Influence of compounds on the ^{3}H-NA uptake by rat brain synaptosomes

	Inhibition of [^{3}H]-NA uptake (in %) (means ± S.E.M. from 6 experiments). Concentration in the incubation medium (M). 10^{-6}	10^{-5}	10^{-4}
DMI	16.53 ± 3.78	32.77 ± 2.35*	82.34 ±5.76†
N-(β-dimethylaminopropionyl) dibenzazepine	26.54 ± 2.81	27.70 ± 4.65*	70.37 ± 6.31†
N-(β-methylaminopropionyl) dibenzazepine	21.37 ± 1.86	26.70 ± 5.27*	75.84 ± 6.24†

In comparison to control (saline instead of compounds): * $P < 0.05$; † $P < 0.01$.

The effect of the drugs was compared with the action of desipramine (DMI)—the most potent inhibitor of the NA uptake among the imipramine-like drugs [14].

All three drugs studied inhibited the [^{3}H]-NA uptake almost to the same degree (Table 1).

The same degree of the inhibitory effect in tertiary and secondary derivatives of β-aminopropionylic derivatives of DBA corresponds to the equal degree of their antagonism with reserpine in the tests of hypothermia and ptosis, revealing the adrenopositive qualities of the studied drugs [8].

Among aminopropylic derivatives of DBA, the secondary analog (DMI) is known to be a more potent inhibitor of NA uptake than the tertiary one—imipramine [1]. However an equal effect of a tertiary and a secondary derivative on NA uptake was found in this study. At the same time the secondary β-aminopropionylic derivative of DBA has a strong inhibitory effect on 5-HT uptake [11] in contrast to the secondary analog of aminopropylic derivatives of DBA [5, 14]. Thus the secondary analog of this β-aminopropionylic derivative of DBA combines the qualities of both imipramine and DMI in relation to 5-HT and NA uptake respectively. Since for the antidepressive action both 5-HT and NA-potentiating effects are important [12], the combination of such qualities in one and the same drug can offer advantages over other antidepressants, selectively inhibiting the 5-HT or NA uptake.

Another advantage of such kind of drugs is connected with the secondary amine group which they contain. Demethylation of tertiary aminopropylic derivatives of DBA is known to be one of the main reactions of their catabolism *in vivo* [2], while demethylation of the secondary compounds proceeds very slowly [7]. Considering the similarity of chemical structures of aminopropylic and β-aminopropionylic derivatives of DBA, the similarity of the main ways of their catabolism *in vivo* may be suggested. In case this assumption is proved, the use of the secondary β-aminopropionylic compounds, inhibiting 5-HT uptake, would carry at least one advantage over tertiary aminopropylic drugs, since the latter lose their ability to inhibit 5-HT uptake after demethylation.

Acknowledgements—I would like to thank Professor J. Knoll and Dr. K. Magyar (Budapest) for helpful advice and encouragement.

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Biochemical Pharmacology, Vol. 28, pp. 939–940.

0006-2952/79/0315-0939 $02.00/0

Beta adrenergically sensitive adenyl cyclase in turkey erythrocytes—Apparent lack of effect on oxygen carriage

(*Received* 26 *April* 1978; *accepted* 14 *July* 1978)

Epinephrine elicits increases in the levels of cyclic-adenosine-3′, 5′-monophosphate (cAMP) in many tissues [1], including turkey erythrocytes. The turkey erythrocyte has a beta-adrenergic receptor on its outer membrane surface [2], which, when stimulated by the *N*-isopropyl analogue of epinephrine (isoproterenol), elicits an increased cytoplasmic concentration of cAMP [3]. These increased erythrocyte cAMP levels are accompanied by the phosphorylation of a specific membrane protein [4] and bi-directional increases in ion fluxes [5]. Since the only known physiological role of the erythrocyte is the carriage of oxygen, we were intrigued by the possibility that a beta-adrenergic-mediated control of oxygen binding may occur in turkey erythrocytes. Accordingly, we set out to determine whether or not beta-adrenergic agents or

cyclic nucleotides stimulated a shift in the oxygen dissociation curve in turkey erythrocytes.

Fresh turkey blood was centrifuged (200 *g*, 30 min, 4°) and the serum and buffy coat were removed. The packed erythrocytes were resuspended and centrifuged three times in buffer (in m-moles: NaCl, 131; KCl, 2; $NaHCO_3$, 24; K_2HPO_4, 1; $CaCl_2$, 1; *d*-glucose, 12; and inositol, 1) and finally resuspended in this buffer to a hematocrit of 25 per cent. Samples were exposed to tonometry for 1 hr at 35° with 95% N_2–5% CO_2 in the presence or absence of *l*-isoproterenol · HCl (10 μM) (Sigma Chemical Co., Boston, MA) or cAMP (2 mM) (Boehringer Mannheim, Germany); then oxygen dissociation curves were obtained using the method of Duvelleroy *et al.* [6]. After tonometry, samples exposed to buffer alone or buffer plus isoproterenol were placed in a boiling water bath for 5 min and cAMP content was measured using the binding assay of Brown *et al.* [7]. Neither 10 μM isoproterenol nor 2 mM of exogenous cAMP caused a significant change in the mean PO_2 at 50% saturation (P_{50}), although exposure to isoproterenol, as noted by previous investigators [2, 3], did result in a significant elevation in cAMP (Table 1).

Table 1. Oxygen binding and cyclic AMP content of turkey erythrocytes

Additions	P_{50}* (mm O_2)	cAMP content (μmoles/1 cells)
None	40.3 ± 0.7	None detected
l-Isoproterenol, 10 μM	40.4 ± 1.4	44.0 ± 4.0
cAMP, 2 mM	38.4 ± 0.3	

* Refers to the partial pressure of oxygen giving 50% hemoglobin saturation. Results represent mean ± S.E.M. of at least three replicate experiments.

The apparent lack of effect of isoproterenol or cAMP on the oxygen dissociation curve in turkey erythrocytes was unexpected. That the beta-adrenergic receptor regulates the oxygen-carrying capacity of these cells was a reasonable expectation based not only on the fact that oxygen carriage is the only known physiological role of the erythrocyte. A consistent observation in turkey [5], pigeon [8] and duck [9] erythrocytes is that beta-adrenergic stimulation elicits increases in ion fluxes in these cells. In what is perhaps the most thorough study to date, Schmidt and McManus [9, 10] have shown that beta-adrenergic stimulation leads to bi-directional increases in Na^+ and K^- fluxes and to a net accumulation of Na^+, K^+, Cl^- and water in duck erythrocytes. These bi-directional increases in ion fluxes led us to expect that a net accumulation of electrolytes and water might also occur in turkey erythrocytes. Chloride accumulation has the potential, by displacing intracellular HCO_3^- (the so-called Hamburger shift), to change intracellular pH and shift the oxygen-dissociation curve of the hemoglobin to the right [11]. Thus, a hypothetical ion flux mechanism was suspected for the regulation of oxygen carriage in avian erythrocytes in response to beta-adrenergic stimulation. Such a mechanism would have, along with other components of the general alarm reaction [12], potential adaptive value. A shift of the oxygen-dissociation curve to the right leads to an increase in the rate of dissociation of oxyhemoglobin in the tissues and, thus, could increase the efficiency of oxygen delivery to tissues.

The theoretical strength of a link between a beta-adrenergic receptor and oxygen carriage in turkey erythrocytes notwithstanding, beta-adrenergic stimulation and increased intracellular cAMP levels did not cause a shift in the oxygen dissociation curve of these cells (Table 1). That beta-adrenergic stimulation, of intensity and duration similar to ours, resulted in increased cation flux in turkey erythrocytes [5] suggests that cation fluxes do not result in changes in intracellular pH. The work of Schmidt and McManus [10] provides a highly likely explanation. They showed that beta-adrenergic stimulation of duck erythrocytes caused a net influx of water as well as Cl. Thus, even though the cell Cl content increased [10], the intracellular Cl concentration may have remained constant and, as a result, there may have been no change in intracellular HCO_3^- concentration and pH.

It is, of course, possible that beta adrenergically mediated ion fluxes in erythrocytes serve yet another purpose. Allen and Rasmussen have shown that beta-adrenergic stimulation of rat and human erythrocytes causes swelling of these cells, an increase in their fragility and rate of spontaneous hydrolysis and a decrease in their filterability [11, 12]. They have suggested that the decrease in filterability might be a mechanism for the control of blood flow through capillaries.

Therefore, even though beta-adrenergic stimulation of turkey erythrocytes led to increases in intracellular cAMP accumulation and membrane ion fluxes, neither beta-adrenergic stimulation nor the direct application of cAMP resulted in any change in the ability of these cells to bind oxygen. Among the explanations for these observations are the possibilities that ion flux changes do not necessarily result in intracellular pH changes and that beta-adrenergic control in turkey erythrocytes regulates something other than oxygen carriage.

Acknowledgements—Supported in part by USPHS grants MH-16674 and MH-31154 and Research Career Development Award NS-00160-01 (M. L. S.).

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