Norepinephrine with its precursors and their antagonists haloperidol and phentolamine interact with dye free radicals in opposite ways

B.S. Marinov

Institute of Biological Physics, USSR Academy of Science, Pushchino, Moscow Region 142292, USSR

Received 2 January 1986

In the reactions with dye free radicals, catecholamines exhibited reversible electron donor and acceptor properties with the effectiveness increasing from tyrosine to norepinephrine. The physiological antagonists haloperidol and phentolamine showed opposite patterns of behaviour in the same reactions, changing their properties as acceptors to electron donors. The regularity observed is similar to that demonstrated earlier by a variety of Na^+ and Ca^{2+} channel modulators.

Ion channel *Flash photolysis* *Dye intermediate* *Electron donor* *Electron acceptor*

1. INTRODUCTION

Transmission of a signal from one nerve to another or from a nerve cell to muscle involves the operation of specific membrane channels known as synaptic channels. The gating of these channels differs from those of Na^+, K^+ and Ca^{2+} which propagate excitation along a nerve cell. The main component of synaptic channel regulation is the action of chemical agents called mediators on the channels, which results in their opening or closing [1]. In this respect, the action of mediators can be compared with the influence of local anesthetics or other modulators on Na^+ or Ca^{2+} channel functioning [2].

The variety of chemical structures of similarly acting agents hampers analysis of the manner in which they affect the channel. This compelled us to look for properties in common among different types of channel regulators that could be related to their action. If such a feature were to be found, it would have even greater significance if its manifestation in agonists and antagonists were of opposite nature.

We succeeded in finding a comparatively simple physico-chemical process in which all the Ca antagonists studied behaved in the opposite manner to Ca agonists. In reactions with dye free radicals, the Ca^{2+} channel blockers felodipine, ryocidil, verapamil and diltiazem exhibited electron donor properties whereas the Ca agonists BAY K 8644 and CGP 28 392 acted as electron acceptors in the same reactions [3]. A number of Na^+ channel agonists and antagonists revealed similar properties [4].

The observed regularity prompted us to investigate the interaction of synaptic mediators and their antagonists with dye free radicals. It was shown that in reactions with excited eosin, norepinephrine acted initially as an electron donor but, when oxidized, behaved as a strong electron acceptor. In contrast, the adrenoblocker haloperidol acted firstly as an acceptor on excited eosin, but became thereafter a vigorous donor. The results support the idea that some common principles should underlie the functioning of channels in excitable tissues and it seems likely that free radical states are involved in channel operation.

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

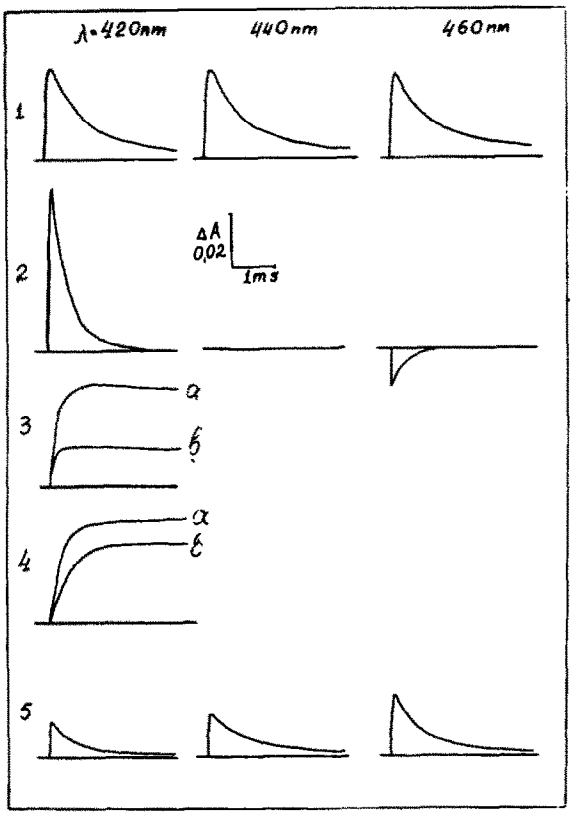

Fig.1. Effects of norepinephrine and haloperidol on the eosin intermediates generated by a flash of light. Panels: 1, eosin (1×10^{-5} M in 0.01 Tris-HCl, pH 8.0); 2, eosin (1×10^{-5} M), norepinephrine (1×10^{-5} M); 3, a – eosin (1×10^{-5} M), NADH (1×10^{-4} M); b – eosin (1×10^{-5} M), NADH (1×10^{-4} M), norepinephrine (1×10^{-5} M); 4, a – eosin (1×10^{-5} M), NADH (1×10^{-4} M); b – eosin (1×10^{-5} M), NADH (1×10^{-4} M), haloperidol (1×10^{-5} M); 5, eosin (1×10^{-5} M), haloperidol (1×10^{-5} M). Deaerated samples; flash light was passed through a GS-18 filter ($\lambda \geqslant 500$ nm).

2. MATERIALS AND METHODS

Pulse and steady light techniques were used as described in [3,4]. Norepinephrine (Fluka), haloperidol and phentolamine (both from Sigma), NADH (Reanal) and eosin (Chimreactive) were used without additional purification. All samples were placed in a Tumberg-type optical cuvette and underwent deaeration by pumping (10^{-3} Torr) for 30 min.

3. RESULTS

Fig.1 illustrates the effects of norepinephrine and its antagonist, haloperidol, on the kinetics of the dye radicals. In the top panel, dye intermediates in the control sample after illumination are shown. At 420 nm triplets and dye anion radicals dominate, whereas at 460 nm triplets together with cation radicals are the dominant components [5]. Within the observed wavelength range, the yield and decay of the intermediates were approximately equal. Norepinephrine enhanced the yield of intermediates and accelerated their decay at 420 nm (panel 2); in the same sample no excited dye intermediates were observed at 440 and 460 nm. Weak negative kinetics at 460 nm can be attributed to dye absorption recovery after bleaching under the light pulse. The disappearance of dye cation radicals, as well as the increase in concentration of anion radicals, demonstrates the electron donor properties of norepinephrine. On the other hand, the acceleration of anion radical decay was consistent with the action of an electron acceptor.

Table 1

Norepinephrine and its precursors dopamine, L-dopa and tyrosine affect the kinetics of eosin intermediates

	Eosin (1×10^{-5} M)	Norepinephrine (1×10^{-5} M)	Dopamine (1×10^{-5} M)	L-Dopa (1×10^{-5} M)	Tyrosine	
					(1×10^{-5} M)	(1×10^{-4} M)
Triplet + anion radical yield (A_{420}, arbitrary units)	33	60	44	55	35	38
Half-life of intermediates at 420 nm (μs)	900	300	300	750	600	400

The usual test for an electron acceptor is the reaction with dye anion radicals generated in excess by addition of NADH to the dye solution. In the control (panel 3, a), the photogenerated dye anion radicals vanished very slowly. Adding norepinephrine greatly diminished the anion radical yield as compared with the control but did not affect the slow kinetics of the remaining anion radicals (panel 3, b).

Similar results to those shown in fig.1, panel 1, were obtained with the norepinephrine precursors, dopamine and L-dopa (table 1). These substances affected the yield and lifetime of the dye anion radicals to a lesser extent at concentrations equimolar with norepinephrine (1×10^{-5} M). Their parent substance, tyrosine, was almost ineffective at 1×10^{-5} M but approached other norepinephrine precursors in effectiveness at 10-fold higher concentration.

In contrast to norepinephrine, its antagonist haloperidol decreased the yield of dye intermediates at all wavelengths tested, the greatest effect occurring at 420 nm (fig.1, panel 5). In the reaction with NADH-generated dye anion radicals, haloperidol only slightly diminished their yield but markedly slowed the rise time (fig.1, panel 4).

The steady light experiments provide another illustration of the properties of norepinephrine and its antagonists. Fig.2 depicts the photoreduction of hemin promoted by the substances under study. In the control, hemin photoreduction was negligible

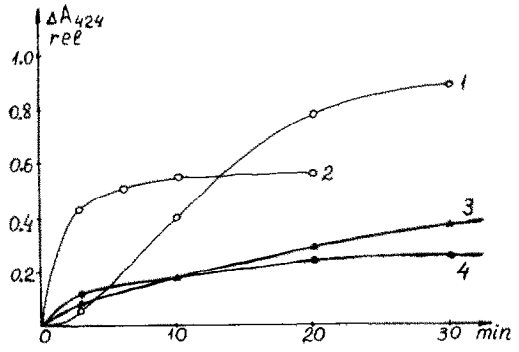

Fig.2. Hemin photoreduction promoted by (1) haloperidol (2×10^{-4} M), (2) norepinephrine (2×10^{-4} M), and (3) phentolamine (2×10^{-4} M) in dimethyl sulfoxide + H_2O mixture (4:1, v/v), sensitized by the eosin derivative erythrosin (1×10^{-5} M). Steady light intensity, 4×10^{14} quanta/s, $\lambda \geqslant 500$ nm. (4) Control.

since the anion and cation dye radicals reacted predominantly with each other. With norepinephrine present, hemin underwent rapid initial photoreduction, which was followed by slowing down, with the amount of reduced hemin approaching asymptotically about one-half of the total amount of hemin in the sample.

With haloperidol, the reaction began at a slow rate and then accelerated, resulting in the complete irreversible reduction of hemin. Another adrenoblocker, phentolamine, also promoted the irreversible photoreduction of hemin but at a markedly slower rate.

4. DISCUSSION

The reaction of an irreversible electron donor, e.g. NADH, with an excited dye resulted in the production of dye anion radicals with an absorption band centered around 420 nm, accompanied by the disappearance of cation radicals with an absorption band around 460 nm (fig.1, panel 3, a). This observation can be represented by the following set of reactions:

$$E \longrightarrow E^x \longrightarrow E^t \quad (1)$$

$$E^t + NADH \longrightarrow E^{\cdot -} + NADH^{\cdot +} \quad (2)$$

$$2E^t \longrightarrow E^{\cdot -} + E^{\cdot +} \quad (3)$$

$$E^{\cdot +} + NADH \longrightarrow E + NADH^{\cdot +} \quad (4)$$

$$2E^{\cdot -} \longrightarrow EH_2 + E \quad (5)$$

where E, E^x and E^t denote eosin in the ground, first singlet and triplet states, respectively.

There was no sign of $NADH^{\cdot +}$ produced in reactions 2 and 4 being involved in other photochemical reactions considered. This could account for the slow disproportionation of dye anion radicals to form colourless EH_2.

The action of norepinephrine on excited eosin was similar to that of NADH in some respects. No positive signal at 460 nm was observed, which would demonstrate the disappearance of dye cation radicals caused by norepinephrine through a process similar to reaction 4. A weak negative signal could be attributed to dye bleaching. The absorption at 420 nm was greatly enhanced, indicating the additional production of dye anion radicals, as for NADH in reaction 2. However, in contrast to the action of NADH, the dye anion

radicals formed by norepinephrine disappeared very rapidly. It seems likely that norepinephrine (NE) behaves firstly as an effective electron donor which becomes a strong acceptor when oxidized:

$$E^t + NE \longleftrightarrow E^{\cdot -} + NE^{\cdot +} \quad (6)$$

Norepinephrine affected the dye intermediates in a concentration-dependent manner up to 10^{-7} M.

In steady light experiments, the high rate of initial photoreduction of hemin caused by norepinephrine illustrates its electron donor properties [4]. As illumination continues and reduced hemin accumulates, its back reaction with oxidized norepinephrine becomes rate determining in the overall process, resulting in saturation of the reduced hemin level:

$$Hemin^{3+} + E^{\cdot -} \longrightarrow hemin^{2+} + E \quad (7)$$

$$Hemin^{2+} + NE^{\cdot +} \longrightarrow hemin^{3+} + NE \quad (8)$$

where $E^{\cdot -}$ and $NE^{\cdot +}$ are formed during process 6. This saturation is evidence of the acceptor properties of oxidized norepinephrine. Hence, the continuous as well as the pulse light experiments characterize norepinephrine as a strong electron donor in the initial reaction with excited eosin, followed by transformation into an acceptor after electron withdrawal.

The action of dopamine and L-dopa on eosin intermediates resembles that of norepinephrine, exhibiting a slightly weaker electron activity at equal concentrations. Tyrosine, the parent substance for the catecholamines studied, showed electron properties similar to those of its derivatives but at concentrations an order higher.

The enhancement of redox activity in catecholamines from tyrosine to norepinephrine correlates with their physiological efficacy. This suggests that the observed electron properties can participate in the mechanisms of neurotransmitter action.

Haloperidol diminishes the quantum yield of dye intermediates at all wavelengths, the greatest effect being at 420 nm. This means that production of anion radicals is depressed by haloperidol to a greater extent than that of cation radicals, this being indicative of the acceptor properties of haloperidol. In more detail, the observed decrease of the yield of intermediates can be explained as the result of reversible reactions of haloperidol with dye triplets:

$$E^t + HP \longleftrightarrow E^{\cdot +} + HP^{\cdot -} \quad (9)$$

At the same time, the oxidation of dye anion radicals by haloperidol further diminishes the optical absorption at 420 nm

$$E^{\cdot -} + HP \longrightarrow E + HP^{\cdot -} \quad (10)$$

In contrast to catecholamines, haloperidol first acted as an electron acceptor, thereafter becoming a donor capable of reducing hemin, as shown in steady light experiments. However, unlike norepinephrine, the initial phase of photoreduction of hemin by haloperidol was rather slow, most probably because of the acceptor properties of haloperidol; with continued illumination, $HP^{\cdot -}$ accumulated resulting in irreversible reduction of hemin. Another adrenoblocker, phentolamine, also promoted complete photoreduction of hemin but at a slower rate.

The results obtained show that neuromediators interact with excited eosin in the opposite manner to their antagonists. This strongly resembles the contrasting redox properties of ion channel blockers and agonists [3,4]. The obvious difference between the catecholamine-type and ion channel regulators is the more complex behaviour of catecholamines and their antagonists: whereas Na^+ and Ca^{2+} channel blockers behave mostly as irreversible electron donors and channel agonists as irreversible acceptors, catecholamines change temporarily from donors to acceptors and vice versa for their antagonists. Such a complexity in the electron activity of catecholamines seems to be in agreement with the current view that catecholamines do not act on channels directly but attach themselves to some protein located near the ion channel and transduce their action through the consecutive changes in cell metabolism [6].

Recently obtained results suggested the possibility of direct action of norepinephrine and dopamine on Ca^{2+} channels. Catecholamine application resulted in a high rate of onset of Ca^{2+} current blocking, which was not mediated by cyclonucleotides or other second messengers [6]. It seems likely that in this case catecholamines revealed their primary electron donor properties analogous with other Ca^{2+} channel antagonists.

The present results have extended the regularity in redox properties observed between ion channel

modulators to the features of synaptic transmission modulators. It appears likely that this observed regularity reflects the possible characteristics of the active working proteins, namely that free radical states constitute the energetics of their functioning, thus forming the basis for the regulation of protein function by electron-active compounds.

REFERENCES

[1] Kehoe, J. and Marty, A. (1980) Annu. Rev. Biophys. Bioeng. 9, 437–465.
[2] Renaud, J.-F., Meaux, J.-P., Romey, G., Schmid, A. and Lazdunski, M. (1984) Biochem. Biophys. Res. Commun. 125, 405–412.
[3] Marinov, B.S. and Saxon, M.E. (1985) FEBS Lett. 186, 251–254.
[4] Marinov, B.S. (1985) FEBS Lett. 191, 159–162.
[5] Kasche, V. and Lindqvist, L. (1965) Photochem. Photobiol. 4, 923–928.
[6] Forscher, P. and Oxford, G.S. (1985) J. Gen. Physiol. 85, 743–764.