Na^+-channel antagonists act as electron donors while agonists act as electron acceptors in reactions with dye free radicals

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The reactions of Na^+-channel antagonists and agonists with dye free radicals were studied by the methods of pulse and continuous absorption spectroscopy. All Na^+ antagonists tested except tetrodotoxin act as electron donors while agonists act as electron acceptors in the reactions with photogenerated dye free radicals. Qualitative correlation between electron donor activity of the Na^+-channel blockers and their electrophysiological potency was observed. The relation of discovered regularity to the Na^+ channel operating was discussed.

Channel *Antagonist* *Agonist* *Dye anion radical* *Photoreduction* *Electron donor* *Electron acceptor*

1. INTRODUCTION

Recently we have found that in the reactions with dye free radicals Ca^{2+}-channel antagonists with diverse chemical structure behaved as electron donors while Ca^{2+} agonists showed electron acceptor properties [1]. It was most striking that a chemical modification converted a dihydropyridine electron donor, nitrendipine, into a Ca^{2+} agonist, BAY K 8644 [2], with electron acceptor features. It suggests that the nature of interaction of channel modulators with free radical states may underlie the drugs' influence on channel functioning.

Here we extended this promising approach to the Na^+-channel modulators. It was found that most of the Na^+-channel blockers acted as electron donors in the reactions with dye radicals, whereas Na^+ agonists served as electron acceptors in analogous processes. A qualitative correlation between electron donor activity of Na^+ antagonists and their channel-blocking potency was observed.

2. MATERIALS AND METHODS

The compounds with electron donor properties enhanced dye anion-radical concentration during illumination of the sample. This was measured directly by a flash-photolysis method by recording transient radical absorption at 420 nm, or as an increased rate of photoreduction of coloured electron acceptors (hemin or myoglobin) in steady light experiments. The sample for flash measurements contained 1×10^{-5} M of the dye eosin (Chemapol). 1×10^{-5} M hemin (Chimreactiv) was added in the steady light experiments. All the samples were deaerated by pumping at 10^{-3} Torr.

In contrast, electron acceptors diminished dye anion-radical concentrations. This was determined by the decrease of anion-radical absorption at 420 nm using the flash technique, or by a dye-bleaching delay promoted by the compound tested. 1×10^{-4} M NADH (Reanal) was added to the samples as a primary electron donor that increased the initial radical concentration and the rate of dye bleaching in the control. Other experimental details were described elsewhere [1,3].

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The compounds tested were: etidocaine, bipuvacaine, trimecaine and GEA 968, all from Astra, *N*-propylajmalin (Giulini Pharma), etacezine (Pharmac. Institute, Moscow), cloramin-T (Fluka), *N*-bromosuccinimide (Sigma), 2,4,6-trinitrophenol (Chimreactiv), veratridine (Aldrich).

3. RESULTS

Fig.1 demonstrates two methods for observation of the electron donor properties of the drugs. In the inset the enhancement of dye anion-radical concentration produced by bipuvacaine was traced by the flash technique. In the control sample that contained only eosin the light flash initially produced triplet species which transformed into dye radicals in the dark (fig.1, inset, trace C).

The triplet's half-life time was about 2 ms, so at the end of the 10 ms sweep only dye anion-radicals would absorb at 420 nm [4]. The ratio of absorption at the end of the 10 ms sweep to the initial absorption (A_{10}/A_0) characterized the relative yield of dye anion-radicals. Bipuvacaine markedly enhanced anion-radical yield in a concentration-dependent manner from 0.22 in the control (trace C) to 0.47 for 5×10^{-4} M (trace a) and 0.90 for 3×10^{-3} M (trace b). Other local anesthetics studied by this method exhibited varying electron-donor activity (table 1) which correlated with their channel-blocking potency (ED_{50}) observed in electrophysiological experiments [5].

Oxidation of dye anion-radicals by hemin resulted in its photoreduction, which can be traced by the change in the hemin Soret band under continuous illumination. The Na^+-channel blocker ajmalin accelerated hemin photoreduction sensitized by the dye erythrosin (fig.1). The initial rate of hemin photoreduction promoted by various Na^+-channel blockers is shown in table 1. Both in the flash and steady light experiments Na^+-channel blockers demonstrated electron-donor properties. The very potent channel blockers etidocaine and *N*-propylajmalin exhibited the strongest electron donor features.

Electron acceptors oxidized photogenerated dye anion-radicals and diminished their concentration. Therefore it is useful to increase the initial anion-radical concentration in the study of electron acceptor features of the drugs. This was achieved by NADH added to the dye solution.

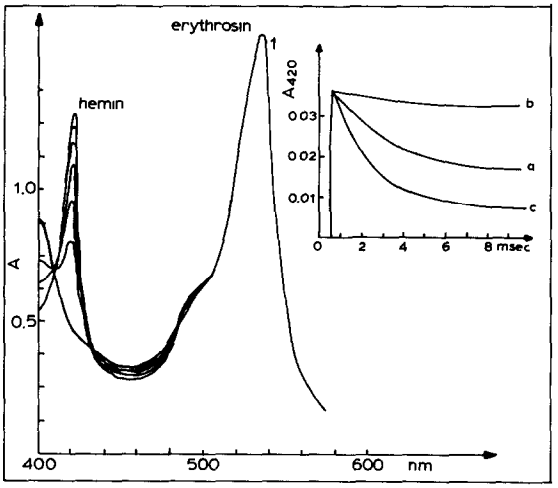

Fig.1. Channel blockers enhance flash-generated dye anion-radical concentration (inset), and promote hemin photoreduction under steady illumination. 1×10^{-4} M *N*-propylajmalin, 2×10^{-5} M erythrosin and 1×10^{-5} M hemin, after 2, 4, 6, 8, 10 and 15 min of illumination. Light intensity, 4×10^{14} quanta/s. Inset: 1×10^{-5} M eosin, alone in the buffer pH = 8.0 (c), with 5×10^{-4} M bipuvacaine (a), or with 1×10^{-3} M bipuvacaine (b).

Table 1

The increase of the relative dye anion-radical yield (A_{10}/A_0) and the relative hemin photoreduction during the initial 2 min of illumination (V_{rel}) promoted by Na^+ antagonists

Antagonist	Concentration (M)	A_{10}/A_0	V_{rel}	ED_{50} (μM)
Control	0	0.22		
Etidocaine	5×10^{-5}	0.80		15
Bipuvacaine	5×10^{-4}	0.47		50
Trimecaine	5×10^{-3}	0.50		250
GEA 968	5×10^{-3}	0.40		600
Tocainide	5×10^{-3}	0.05		500
N-Propyl ajmalin	1×10^{-4}		0.33	
Bipuvacaine	1×10^{-4}		0.09	
Etacezine	1×10^{-4}		0.04	
Procaine	2×10^{-4}		0.05	
Benzocaine	1×10^{-3}		0.03	

Experimental conditions as in the legend to fig.1

In the control without drug pulse illumination produced maximal amount of dye anion-radicals, their concentration remaining nearly constant during the 10 ms sweep (fig.2, inset, trace C). When the sample contained the Na^+-channel agonist veratridine [6] the first flash revealed fast disappearance of anion-radicals (fig.2, inset, trace 1). The next flashes gradually restored the initial anion-radical kinetics. It seems most likely that veratridine became more reduced in the course of illumination and that it shifts the redox conditions in the sample in the less oxidizing direction.

Other Na-agonists tested, 2,4,6-trinitrophenol [7], chloramin-T [8] and *N*-bromosuccinimide [9] influenced the anion-radical kinetics in a similar way though at different concentrations of the drugs (table 2). Trinitrophenol exhibited the strongest electron-acceptor properties, already accelerating radical disappearance markedly at 5×10^{-6} M. Veratridine at 5×10^{-4} M produced a similar effect, and both cloramin-T and *N*-bromosuccinimide acted at 1×10^{-3} M.

In the control sample with NADH the dye undergoes fast bleaching under continuous illumination as a result of the high anion-radical concentration formed (fig.2, C). As expected for electron acceptor action, veratridine slowed the initial phase of the dye bleaching kinetics in a concentration-dependent manner (fig.2, a and b). When a part of the veratridine became reduced the bleaching rate was increased and finally reached the control value, in agreement with the flash experiments.

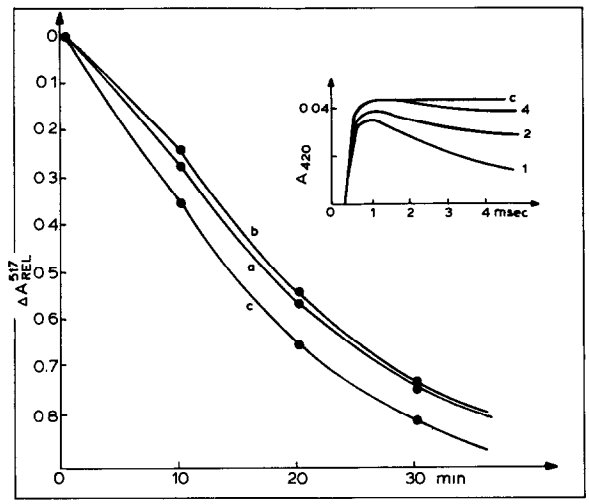

Fig.2. The influence of veratridine on the initial rate of dye bleaching and on the dye anion-radical kinetics (inset). 1×10^{-5} M eosin, 1×10^{-4} M NADH, veratridine at (a) 5×10^{-4} M, (b) 1×10^{-3} M, (c) control. Inset: 1×10^{-5} M eosin and 1×10^{-4} M NADH (c, control), and with 5×10^{-4} M veratridine after 1, 2 and 4 flashes.

Table 2

Second order rate constants for dye anion-radical quenching by Na^+-channel agonists

	Trinitro-phenol	Veratri-dine	NBS	Chlora-min-T
K ($M^{-1} \cdot s^{-1}$)	2×10^8	7×10^5	1×10^6	1×10^6

Among the Na^+-channel modulators studied only tetrodotoxin, a highly specific Na^+-channel blocker, did not reveal noticeable electron-donor or -acceptor features. This suggests that its mechanism of channel blocking is different from the other antagonists.

4. DISCUSSION

The main result of this work is the finding that Na^+ antagonists exhibited common properties as electron donors while Na^+ agonists behaved as electron acceptors in a simple physico-chemical process. In the reactions with dye radicals the antagonists and agonists showed opposite action as is characteristic for their behaviour in physiological experiments. It is essential that this regularity was shown by Na^+ agonists and antagonists with highly heterogeneous chemical structures. Within the chemically more homogeneous tertiary amine local anaesthetics group the electron donor capacity decreased in the sequence etidocaine > bipuvacaine > trimecaine > GEA 968, which is in line with the drug activity. A similar correlation was observed between ajmalin derivatives and bipuvacaine derivatives (not shown). Some local anaesthetics (benzocaine, tocainide) exhibited weak electron donor properties in disagreement with their blocking potency. It is likely that other drug features such as hydrophobicity and specificity of binding on the receptor sites may modulate the local concentration of the drug near the channel.

We believe that the observed electron donor or acceptor activity of Na^+-channel modulators underlies the nature of their interaction with the channel forming protein. The different chemical structures of the antagonists studied speak against a common receptor site on the channel. It must be supposed that modulators exert their common action on the channel at different sites. If the blockers acted at different sites it is doubtful that they would promote the same conformational change of the channel protein simply as a result of their binding. It is more likely that protein conformational changes that are associated with closing or opening the channel are a result of the influence of the modulators on the gating mechanism. The observed regularity, namely that all channel blockers acted as electron donors while agonists are electron acceptors, prompted us to suppose that free radical states arise in the channel-forming protein during its gating function. This conception is supported partly by the observation that the globular moiety of various proteins may mediate electron transfer from free-radical donors to acceptors [3]. Some theoretical considerations predicted the participation of unpaired electrons in channel operation [10]. It is interesting that both Ca^{2+} modulators [1] and Na^+ modulators showed similar behaviour in photochemical reactions.

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