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LUMINOL- AND LUCIGENIN-DEPENDENT CHEMILUMINESCENCE DURING AUTOOXIDATION OF 6-HYDROXYDOPAMINE

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Endogenous 6-hydroxydopamine (6-OHDA), a minor product of secondary metabolism of dopamine, is one of the most powerful neurotoxins, for it can disturb the structural and functional integrity of the neuronal membranes of the brain [5, 6]. The neurotoxicity of 6-OHDA is linked primarily with its ability to accumulate in large amounts in the central and peripheral catecholaminergic neurons, damaging them and causing the development of "parkinsonism" in experimental animals [3, 4]. It has been demonstrated on model systems and systems in vitro that one of the most likely mechanisms for the realization of the pathological action of 6-OHDA is its ability to undergo autooxidation, with the formation of O_2^- and H_2O_2, which are powerful activators of free-radical oxidation of membrane lipids and proteins. Indeed, the fact that 6-OHDA can activate lipid peroxidation (LPO) of brain membranes was demonstrated recently [7, 10]. However, stimulation of LPO of neuronal membranes by autooxidation of 6-OHDA has a number of special features: first, the stimulating effect depends ultimately on the 6-OHDA concentration; second, superoxide dismutase (SOD) which, as we know, usually inhibits LPO in biomembranes, potentiates 6-OHDA-induced LPO of brain membranes [7]. In this connection, in order to study the mechanism of initiation of LPO under the influence of 6-OHDA, the features of its autooxidation were studied by the luminol- and lucigenin-dependent chemiluminescence (ChL) method.

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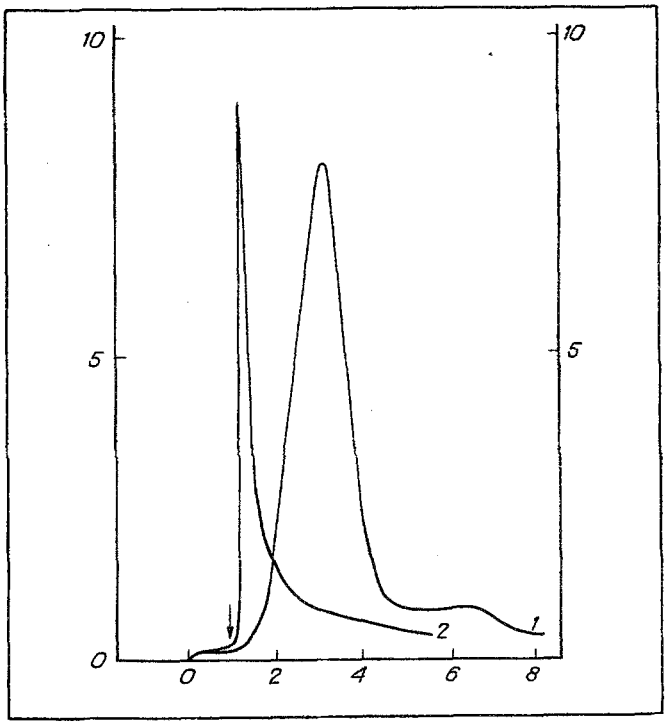

Fig. 1. Kinetic curves of luminol- (1) and lucigenin-dependent (2) ChL, accompanying autooxidation of 6-OHDA. 6-OHDA was added from a solution with pH 4.0; arrow indicates time of addition of 6-OHDA at pH 11.0. Concentrations of luminol and lucigenin $5 \cdot 10^{-5}$ M. Ordinate, intensity of chemiluminescence (in relative units). Abscissa, time (in min).

EXPERIMENTAL METHOD

ChL arising during autooxidation of 6-OHDA was recorded on a commercial 1250 luminometer (LKB, Sweden). All measurements were made at room temperature with constant stirring. The reaction was initiated by addition of 6-OHDA to the cuvette in appropriate concentrations (6-OHDA was dissolved in buffer, pH 4.0). The intensity of ChL was calculated from the zero line to the maximum of the peak of the kinetic curve of activated ChL and was expressed as a percentage. The following reagents were used: lucigenin and 6-OHDA from "Sigma," luminol from "Serva."

EXPERIMENTAL RESULTS

In a series of experiments Cohen [3-5] found that O_2^- is an intermediate during autooxidation of 6-OHDA. The most highly sensitive method of recording O_2^- is well known to be ChL in the presence of special chemiluminescent probes and, in particular, luminol and lucigenin [1, 2]. In fact, as the results given in Fig. 1 show, autooxidation of 6-OHDA at alkaline pH values is accompanied by luminol- and lucigenin-dependent ChL. The maximal intensity of ChL recorded in the presence of lucigenin, a specific ChL-probe for O_2, clearly was 1000 times greater than in the presence of the equivalent quantity of the nonspecific ChL-probe, luminol. This fact is in good agreement with the difference in constants of interaction of O_2^- with luminol and lucigenin. The kinetics of ChL in both cases contains only one component: a rapid flash, which develops instantaneously after addition of the 6-OHDA solution, for lucigenin-dependent ChL and a slower flash with maximum of ChL at the 1st-2nd minute for luminol-dependent ChL. The results are in good agreement with existing data on kinetic parameters of luminol- and lucigenin-dependent ChL in systems generating O_2^- [1, 2].

The conclusion that luminol- and lucigenin-dependent ChL, accompanying autooxidation of 6-OHDA does in fact reflect O_2^- formation also was confirmed by analysis of dependence of the intensity of ChL of the 6-OHDA solution on pH of the incubation medium (Fig. 2). Clearly within the pH range from 4.0 to 9.0, luminol- and lucigenin-dependent ChL of the 6-OHDA solution was virtually absent, whereas at higher pH values the intensity of ChL rose sharply. This effect can

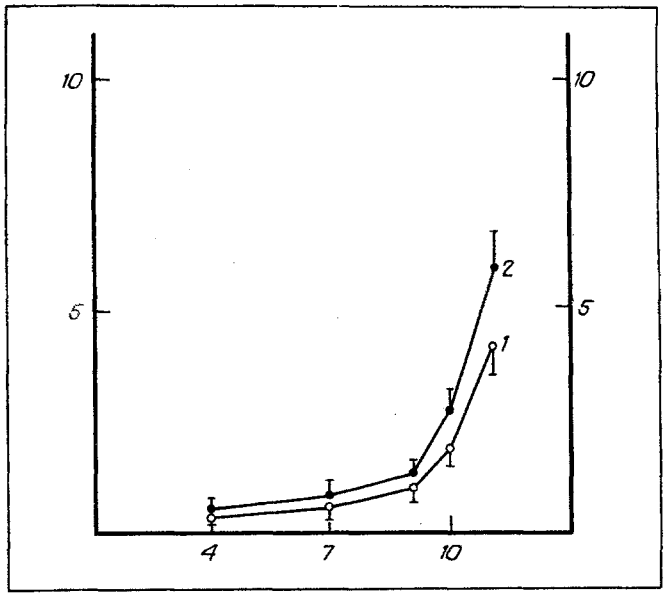

Fig. 2. Effect of pH of incubation medium on luminol- (1) and lucigen-independent (2) ChL of 6-OHDA solution. Same conditions as in Fig. 1. Ordinate, intensity of chemiluminescence (in relative units); abscissa, pH.

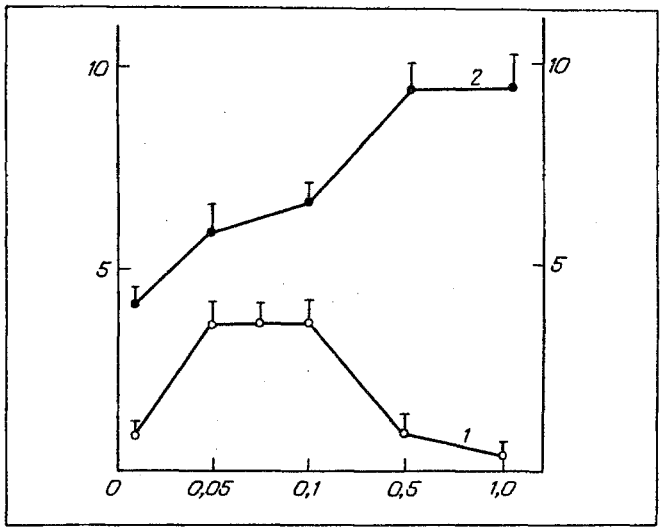

Fig. 3. Intensity of luminol- (1) and lucigenin-dependent (2) ChL of 6-OHDA solution as a function of 6-OHDA concentration at pH 11.0. Same conditions as in Fig. 1. Ordinate, intensity of chemiluminescence (in relative units); abscissa, concentration of 6-OHDA (in mM).

be explained in terms of an increase in the life span of O_2^- at alkaline pH values [8,9]. Thus the data as a whole lead to the conclusion that luminol- and lucigenin-dependent ChL reflects O_2^- generation during spontaneous oxidation of 6-OHDA.

The suggestion was put foward previously that the O_2^- formed is responsible for the chain character of 6-OHDA autooxidation [3-5]. Hence it follows that besides its interaction with ChL-probes, O_2^- may also react with the 6-OHDA molecule. It can accordingly be postulated that in the 6-OHDA/luminol and 6-OHDA/lucigenin systems used in this investigation, "competition" for O_2^- ought to exist between the ChL-probes and 6-OHDA. This hypothesis was confirmed

by the characteristic plateaulike dependence of the intensity of luminol- and lucigenin-dependent ChL of the 6-OHDA solution on the 6-OHDA concentration (Fig. 3). The shift of part of the plateau of intensity of lucigenin-dependent ChL by an order of magnitude to the right compared with luminol-dependent ChL is most probably attributable to the difference in velocity constants of the reaction of O_2^- with luminol and with lucigenin.

It can thus be concluded from all the data obtained by the ChL method that autooxidation of 6-OHDA proceeds in accordance with a radical mechanism, the "central" intermediate of which is O_2^-. This conclusion regarding the intermediate role of O_2^- during autooxidation of 6-OHDA is in good agreement with data obtained previously on the extremal character of 6-OHDA-induced LPO of neuronal membranes [7]. In the light of the results of the present investigation, the potentiating effect of SOD in 6-OHDA-stimulated peroxidation of synaptosomes also becomes understandable. In fact, considering that catalase in the brain is found only in trace amounts, the shift of equilibrium in the reactions of 6-OHDA toward hydrogen peroxide (H_2O_2) formation in the presence of metals of variable valency ought to stimulate generation of the hydroxyl radical ($OH^{\cdot}$) and, consequently, ought to initiate LPO.

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