Inhibition of the sodium and potassium-stimulated adenosine triphosphatase activity during autooxidation of apomorphine

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Apomorphine acts fairly selectively to stimulate the dopaminergic receptors (Andén, Rubenson & others, 1967; Ernst, 1967). Owing to this property it represents an important pharmacological tool in the examination of dopaminergic systems. As a result of an ever more intensive interest in the dopaminergic receptors in the central nervous system an increasing number of papers have discussed also the *in vitro* biochemical effects of apomorphine (e.g. Goldstein, Freedman & Backstrom, 1970; Kebabian, Petzold & Greengard, 1972; Di Chiara, Balakleevsky & others, 1974; Ferris, Tang & Russell, 1975; Bucher & Schorderet, 1975; Miller, Kelly & Neumeyer, 1976). However, the experiments now reported clearly show that in *in vitro* systems apomorphine's oxidation results in inhibition of Na^+, K^+-ATPase activity that might lead to the formation of various artifacts. Moreover, this *in vitro* effect of apomorphine might be of value in studying the inhibitory mechanisms of Na^+, K^+-ATPase activity.

CFE rats of either sex, 150–250 g, were used. From the whole brains a Na^+, K^+-ATPase rich fraction was obtained by the cell fractionation procedure of Skou (1962) with the alterations of Schaefer, Seregi & Komlós (1974). Samples of the particulate suspension containing 20–30 μg of protein were assayed for ATPase activity in 1 ml incubation mixtures. The standard incubation system for determination of the total ATPase activity contained 50 mM tris-HCl buffer pH 7·4, 3 mM $MgCl_2$, 100 mM NaCl, 30 mM KCl, 3 mM Na_2ATP (Sigma, St. Louis). Mg^{2+}-ATPase activity was measured in a similar reaction mixture with the omission of NaCl and KCl and containing 0·5 mM ouabain (Fluka, Buchs). Various amounts of apomorphine hydrochloride (Ph. Hg. VI.) were tested for their effect on ATPase activities in the presence or absence of 0·1 mM of L-ascorbic acid (Merck, Darmstadt) or L-cysteine (Reanal, Budapest) and 0·2 mM of EDTA (Reanal, Budapest). Determination of the ATPase activity was based on the measurement of inorganic phosphate liberated from ATP. The method of Fiske & Subbarow (1925) was adopted to measure inorganic phosphate. Incubations were carried out and the enzyme activities were calculated as described previously (Schaefer, Seregi & Pfeifer, 1973). Protein was determined according to Lowry, Rosebrough & others (1951).

Fig. 1 shows the ATPase activities of the partially purified Na^+, K^+-ATPase preparation, isolated from rat brain, in the presence of various amounts of apomorphine. Na^+, K^+-ATPase was strongly inhibited (50 % inhibition was at about 2×10^{-5} M of apomorphine), while the Mg^{2+}-ATPase activity was inhibited to a slight extent only. Similar differences were found also in the sensitivities of Na^+, K^+- and Mg^{2+}-ATPase activities when experiments were made on microsomal preparations rich in Mg^{2+}-ATPase activity.

The strong inhibitory effect of apomorphine on Na^+, K^+-ATPase activity is eliminated by the presence of ascorbic acid or cysteine in the incubation mixture (Fig. 2). Moreover, even the slight increase of Na^+, K^+-ATPase activity could be observed. The experiment was made in the presence of 0·2 mM EDTA because of the inhibitory action of ascorbic acid on Na^+, K^+-ATPase activity in the absence of EDTA (Schaefer & others, 1974). 0·2 mM EDTA did not affect the inhibitory effect of apomorphine.

Apomorphine is readily oxidized and the effects of reducing agents such as ascorbic acid or cysteine suggest that the inhibitory effect of the drug on Na^+, K^+-ATPase activity must be related to its spontaneous oxidation. Accordingly, the inhibition of Na^+, K^+-ATPase activity might occur following the oxidation of apomorphine, or, as with phenothiazines, as a result of the free radical intermediates formed during oxidation (Akera & Brody, 1968). Certain reactive products formed from O_2 during apomorphine oxidation might also be responsible for the inhibition of

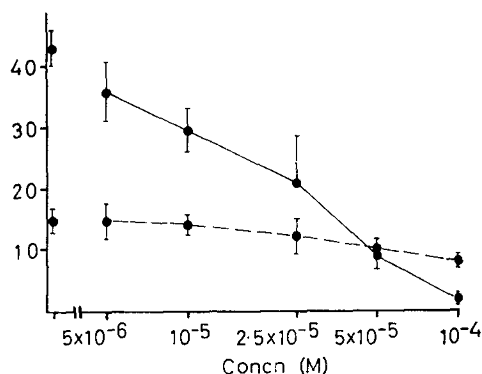

FIG. 1. Inhibition of the ATPase activities of the partially purified Na^+, K^+-ATPase preparation isolated from rat brain in the presence of apomorphine (M). Continuous line: Na^+, K^+-ATPase activity; broken line: Mg^{2+}-ATPase activity. Means of three experiments in duplicate ± s.e. y axis-μmol Pi mg^{-1} protein per 15 min.

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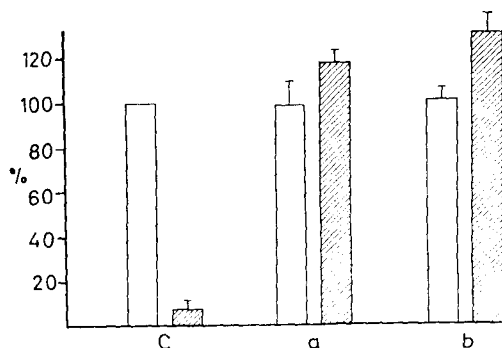

FIG. 2. Antagonization of the inhibitory action of apomorphine (10^{-4}M) on Na^+, K^+-ATPase activity by b, L-ascorbic acid (10^{-4}M) and a, L-cysteine (10^{-4}M). The experiments were carried out in the presence of 0·2 mM EDTA. Results are the means of three experiments in duplicate ± s.e. Hatched columns in the presence of apomorphine. Open columns controls. C-Control. y axis-Na^+, K^+-ATPase activity (% of control).

enzyme activity. In relation to this third possibility, we have examined the effect of 6-hydroxydopamine on ATPase activities, since superoxide and hydroxyl radicals are produced during the autooxidation of this compound (Cohen & Heikkila, 1974). However, up to a concentration of 10^{-4} M no alteration in ATPase activity was found. Apomorphine might therefore serve as an interesting model compound for the study of the inhibitory mechanisms exerted on Na^+, K^+-ATPase activity.

The oxidation of apomorphine and thus the inhibition of Na^+, K^+-ATPase activity does not seem to occur *in vivo*, possibly because of the antagonizing effect of the endogenous reductive agents studied. The concentration of ascorbic acid e.g. in the brain is mM (Rajalakshmi & Patel, 1968; Schaefer & others, 1974). Whenever apomorphine is present, the inhibition of Na^+, K^+-ATPase activity in all *in vitro* systems, where no sufficient reductive capacity can be assured should be borne in mind. The inhibition of Na^+, K^+-ATPase activity might lead to the damage of various transport processes, e.g. the uptake of transmitters by the nerve endings (Bogdanski, Tissari & Brodie, 1968; White & Keen, 1971; Prakash, Fontana & Henkin, 1973). Neither can the possibility be excluded that other enzymatic processes are also inactivated during the oxidation of apomorphine.

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