EFFECT OF MORPHINE in vitro ON OXIDATIVE PHOSPHORYLATION IN RAT LIVER MITOCHONDRIA

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Experiments in vitro on rat liver mitochondria showed that morphine inhibits oxidation of substrates in the presence of ADP and reduces the rate of phosphorylation and the activity of dinitrophenol-stimulated ATPase. The inhibition constant for all these reactions is the same, namely 6.5 mM. Respiration of mitochondria in the presence of an uncoupling agent and also the activity of ATP synthetase and ATPase of the submitochondrial particles are not inhibited in the presence of morphine. It is postulated that morphine inhibits the transfer of adenine nucleotides across the mitochondrial membrane.

KEY WORDS: liver mitochondria; oxidative phosphorylation; ATPase; morphine.

Administration of morphine produces hypothermia in an animal [3], lowers the ATP level in the brain [4], inhibits contractions of smooth muscle [8], modifies the transport of glucose into cells [5], and changes the activity of glycolysis [2]. These effects of morphine can be presumed to be the result of its influence on oxidative phosphorylation in the mitochondria.

The object of this investigation was to study the effect of morphine on the mitochondrial system of oxidative phosphorylation.

EXPERIMENTAL METHOD

Experiments were carried out on liver mitochondria of male albino rats and on phosphorylating submitochondrial particles (PSPs)* of bovine heart. Mitochondria were isolated in a medium containing 0.3 M sucrose and sedimented under the same conditions. Mitochondrial preparations with a protein concentration of 120-140 mg/ml were used.

The oxygen consumption was measured polarographically by means of an open steady-state platinum electrode. Activity of ATPase and ATP synthetase was measured potentiometrically. The platinum and glass electrodes with the electrodes for comparison were mounted in a single block so that the oxygen consumption and changes in pH could be recorded simultaneously. The volume of the cuvette was 2 ml.

The incubation medium for recording the oxygen consumption and ATP synthetase activity contained 0.3 M sucrose, 0.02 M KCl, 0.005 M $MgCl_2$, 0.003 M KH_2PO_4, 0.002 M N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES-buffer), 0.005 M succinate, 10^{-6} M rotenone, and 0.2 mM ADP. The incubation medium for measuring ATPase activity contained 0.3 M sucrose, 0.02 M KCl, 0.005 M $MgCl_2$, 0.004 M HEPES-buffer, and 0.004 M ATP; the reaction was started by the addition of 2,4-dinitrophenol (DNP) up to a concentration of 25 μM. Activity of PSP ATP synthetase was measured on the MPS-3 (Hitachi) spectrofluorometer from the rate of reduction of $NADP^+$ in a coupled glucose hexokinase and glucose-6-phosphate

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TABLE 1. Effect of Morphine on Respiration Rate of Mitochondria and ATP Synthetase and ATPase Activity of Submitochondrial Particles (M±m)

Condition of incubation	Respiration rate of mitochondria (in n atoms O_2/mg protein/min)		SMP	
	in the presence of 200 μM ADP	in the presence of 25 μM DNP	ATP synthetase activity (in μmoles $NADP \cdot H_2$/mg protein/min)	ATPase activity (in μmoles H^+/mg protein/min)
Without morphine	77±2	112±3	273±7	66±3
Morphine (5mM)	60±3	110±2	260±11	70±3

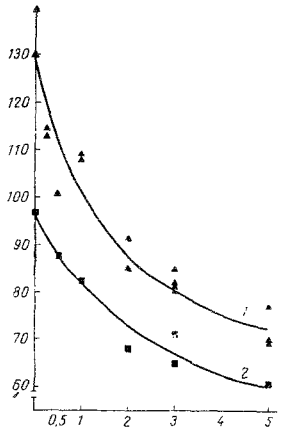

Fig. 1

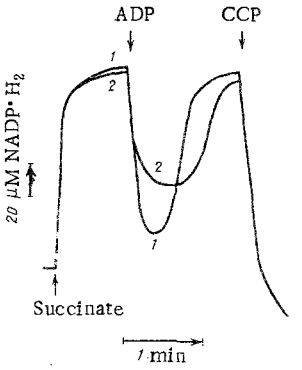

Fig. 2

Fig. 1. Effect of morphine on ATP synthetase activity (1) and ATPase activity (2) of rat liver mitochondria. Abscissa, morphine concentration (in mM); ordinate, ATP synthetase or ATPase activity (in μmoles H^+/mg protein/min).

Fig. 2. Effect of morphine on reversed electron transport in respiratory chain of rat liver mitochondria: 1) without morphine; 2) in presence of 5 mM morphine. Added: 5 mM succinate, 200 μM ADP, 1 μM CCP (m-chlorocarbonyl cyanide phenylhydrazone).

dehydrogenase system [6]. The incubation medium contained 0.3 M sucrose, 0.02 M KCl, 0.005 M $MgCl_2$, 0.003 M KH_2PO_4, 0.002 M HEPES-buffer, 0.015 M glucose, 0.3 mM $NADP^+$, 0.005 M succinate, 10^{-6} M rotenone, 30 units hexokinase, 0.46 units glucose-6-phosphate dehydrogenase, and 0.2 mM ADP. Reversed electron transport in the respiratory chain of the mitochondria from succinate to NAD^+ was measured from the rate of reduction of endogenous pyridine nucleotides (PN) on the spectrofluorometer. The incubation medium was the same as for measurement of the oxygen consumption, except that rotenone was excluded. The volume of the cuvette for the fluorometric measurements was 3 ml. The wavelength of the exciting light was 340 nm and the wavelength of the fluorescence 456 nm.

Morphine hydrochloride solution, neutralized to pH 7.5, was used in the experiments in concentrations of 0.5 to 5 mM depending on the objects of the experiment.

All measurements were carried out at 20°C and pH 7.5.

EXPERIMENTAL RESULTS

Measurement of the respiration of the mitochondria in different states showed that morphine inhibits oxidation of succinate in the presence of ADP (phosphorylating oxidation) but does not inhibit electron transfer in an uncoupled respiratory chain (in the presence of DNP) (Table 1). It can accordingly be concluded

that the inhibitory action of morphine is connected with inhibition of energy transport reactions and not with inhibition of electron transport in the respiratory chain. Morphine likewise is not an uncoupler, for the respiration rate of mitochondria in Chance's 4th state is not increased in the presence of morphine.

The effect of morphine on ATPase and ATP synthetase activity of the mitochondria is shown in Fig. 1. During oxidative phosphorylation in the presence of succinate, alkalification of the medium takes place only through ATP synthesis from ADP and phosphate and the liberation of a proton into the medium is observed in the ATPase reaction. As Fig. 1 shows, both reactions are inhibited equally by morphine. Calculation of the inhibition constants from these data by Dixon's method [1] showed their complete agreement: the value of K_i for ATPase and for the ATP synthetase system was 6.5 mM.

The adenylate kinase activity of the mitochondria, measured fluorometrically from the reduction of NAP^+ in a hexokinase–glucose-6-phosphate dehydrogenase system [6], was unchanged in the presence of morphine; inhibition of oxidative phosphorylation was thus unconnected with activation of the adenylate kinase reaction in the mitochondria.

The kinetics of reduction of PN in the mitochondria on reversal of electron transport in the presence of succinate is illustrated in Fig. 2. The addition of ADP causes cyclic oxidation and reduction of PN, connected with activation of the transfer of endogenous high-energy compounds to ADP and the formation of ATP, accompanied by an increase in the rate of electron transport (removal of respiratory control). On exhaustion of ADP in the incubation medium, reduction of PN again takes place. In the presence of morphine (curve 2) inhibition of the rate of PN reduction is not observed, i.e., the rate of formation of high-energy compounds and the reversed electron transport from succinate, dependent on them, is not reduced. However, the addition of ADP causes a much lesser degree of oxidation of PN and an increase in the time required for phosphorylation of the added ADP and subsequent reduction of PN. The rate of oxidation of PN on the addition of the uncoupling agent CCP was the same in the presence and absence of morphine. It can be concluded from these findings that morphine does not inhibit the electron transport chain or the reaction of formation of the high-energy intermediate compound at the coupling points, but in all probability it prevents the penetration of ADP to the enzymes of the inner mitochondrial membrane responsible for ATP formation.

This hypothesis was confirmed by investigation of the effect of morphine on the ATPase and ATP synthetase activity of the SMP (Table 1). In these preparations ATP synthesis by oxidative phosphorylation and ATP hydrolysis in the presence of the uncoupling agent were unconnected with the stage of adenine nucleotide transport through the mitochondrial membrane, for these reactions are carried out by enzymes located on the outer surface of the SMP vesicles. As will be clear from Table 1, both enzymes are insensitive to morphine.

It can thus be concluded from the facts presented above that morphine inhibits the transport of adenine nucleotides through the mitochondrial membrane and thereby inhibits the activity of oxidative phosphorylation. Considering that morphine can accumulate in the cells of some tissues against the concentration gradient [7], it can be postulated that a high morphine concentration leads to de-energization of the tissue through inhibition of oxidative phosphorylation in the mitochondria.

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