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European Journal of Pharmacology 276 (1995) 101–105

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7-Oxo-prostacyclin affects the electrogenic Na^+/K^+ pump in mouse diaphragm fibers

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Received 9 August 1994; revised 31 December 1994; accepted 3 January 1995

Abstract

The effect of a stable prostacyclin derivative, 7-oxo-prostacyclin, on the electrogenic Na^+/K^+ pump in mouse diaphragm muscle was investigated. Resting membrane potentials of muscle cells were measured with glass microelectrodes. In fresh diaphragm preparations from 7-oxo-prostacyclin-pretreated mice (50 $\mu g \cdot kg^{-1}$ 7-oxo-prostacyclin i.p. 30–34 h prior to the experiment) resting membrane potentials were significantly higher (-83.86 ± 1.40 mV, mean ± S.E.M.) than in control preparations (-70.78 ± 1.07 mV). Ouabain at a concentration of 10^{-4} mol $\cdot l^{-1}$ abolished this hyperpolarization ($P < 0.05$). The electrogenic effect induced by addition of 5 mmol $\cdot l^{-1}$ K^+ to Na^+-loaded muscles from 7-oxo-prostacyclin-pretreated animals was also enhanced (Δ resting membrane potential = 21.73 ± 3.13 mV) compared with that of the controls (Δ resting membrane potential = 18.36 ± 1.29 mV). Ouabain antagonized the increase in electrogenicity of Na^+-loaded diaphragms induced by 7-oxo-prostacyclin pretreatment ($P < 0.05$). In contrast to control preparations, no inhibition of the electrogenic effect induced by 10 mmol $\cdot l^{-1}$ Ca^{2+} ions in Na^+-loaded muscles was observed in diaphragms from 7-oxo-prostacyclin-pretreated animals. In acute experiments with Na^+-loaded muscles, where 10^{-7} mol $\cdot l^{-1}$ 7-oxo-prostacyclin was added to the bath, resting membrane potential reached up to -100 mV. The electrogenic pump-induced increase in resting membrane potential amounted to approximately 30 mV. This effect could be also abolished by 10^{-4} mol $\cdot l^{-1}$ ouabain ($P < 0.05$). The data indicate a stimulating effect of 7-oxo-prostacyclin on the electrogenic Na^+/K^+ pump and increased tolerance of the skeletal Na^+/K^+ pump to ouabain and Ca^{2+}.

Keywords: 7-Oxo-prostacyclin; Na^+/K^+ pump; Muscle fiber

1. Introduction

The sodium salt of 7-oxo-prostacyclin exhibits enhanced molecular stability in aqueous solution (Szekeres et al., 1983). The substance (in a dose of 50 $\mu g \cdot kg^{-1}$ i.p.) provides substantial protection against both the loss of intracellular K^+ and the gain of Na^+ induced by acute coronary occlusion, and the accumulation of Ca^{2+} induced by subsequent reperfusion of the myocardium (Szekeres et al., 1988,1989). This suggests that this drug may act on the membrane Na^+/K^+ pump (E.C. 3.6.1.3) which stabilizes the intracellular Na^+ and K^+ levels.

In our study we investigated if 7-oxo-prostacyclin affects the skeletal muscle Na^+/K^+ pump activity (a physiological equivalent of (Na^+/K^+)-ATPase, Skou, 1975) in diaphragm fibers. Electrogenic sodium pump activity can be estimated in the model of sodium-loaded diaphragm muscle fibers (Kernan, 1962). The effect of 7-oxo-prostacyclin was studied: (a) on preparations from pretreated animals and (b) during direct addition of 7-oxo-prostacyclin to the muscle bath.

2. Materials and methods

All experiments were performed in accordance with the Declaration of Helsinki and internationally accepted principles for the care and use of experimental animals. Female mice (21 ± 1 g body weight) were killed by cervical dislocation. After excision the di-

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SSDI 0014-2999(95)00017-8

aphragm was transferred to Krebs solution as modified for diaphragms by Liley (1956), containing (in mmol · l^{-1}): Na^+ 149.8; K^+ 5.0; Ca^{2+} 2.0 or 10.0; Mg^{2+} 1.0; HCO_3^- 12.6; $H_2PO_4^-$ 1.0; glucose 11.0; pH = 7.2. The latter solution enabled us to adjust the Ca^{2+} concentration of the incubation medium to values as high as 10 mmol · l^{-1}. After isolation, the diaphragms were either directly used for experiments, or they were preincubated for 4–6 h in a K^+-free solution gassed with a mixture of 95% O_2 and 5% CO_2 at room temperature (21 ± 1°C). At this temperature the Na^+/K^+ pump is functional and the time course of the hyperpolarization permits electrophysiological measurements. This incubation resulted in an increase in intracellular sodium concentration from 15 to 30 mmol · l^{-1} (Dlouhá et al., 1981) and in a corresponding decrease in K^+ concentration (Zemková et al., 1982) and induced a decrease in the resting membrane potential by 10–15 mV (Vyskočil et al., 1985). The electrogenic activity of the sodium pump was identified as a temporal hyperpolarization occurring upon the addition of 5 mmol · l^{-1} K^+ to the 'Na^+-loaded' diaphragm muscle preparation (Kernan, 1962; Dlouhá et al., 1981). Resting membrane potentials were measured on superficial muscle fibers by repeated insertion of glass microelectrodes filled with 3 mol · l^{-1} KCl. A high-impedance preamplifier was used for the recordings (Fatt and Katz, 1951; Ujec, 1988).

2.1. Drug addition

In electrophysiological experiments the diaphragms from 7-oxo-prostacyclin-pretreated or non-pretreated animals were used. The drugs were added into Liley K^+-free medium for 5 min (in Na^+-loaded or fresh diaphragms isolated from pretreated animals) or 20 min (in acute experiments with diaphragms from non-pretreated animals). Then 5 mmol · l^{-1} K^+ was added to the medium bathing the diaphragm and initiated Na^+/K^+ pump activity. The reversibility of the drug-induced effect was examined during the washout procedure with new Liley solution.

The animals were pretreated by 7-oxo-prostacyclin administered intraperitoneally in saline solution in a dose of 50 μg · kg^{-1} 30–34 h before the experiment.

2.2. Chemicals

7-Oxo-prostacyclin was a gift from Prof. Dr. Szekeres of Szeged (Hungary). Ouabain was purchased from Fluka (Switzerland), and the rest of the chemicals originated from BDH (UK). All substances used were of analytical grade.

2.3. Statistics

Statistical evaluation of experimental data (differences between the control and 7-oxo-prostacyclin-pretreated groups) was performed by means of a one-way analysis of variance (Egermayer and Boháč, 1984; Dahlberg, 1948). For Student's *t*-test the differences were considered significant at $P < 0.05$. Each experimental group consisted of 3 diaphragms. The total numbers of fibers penetrated in each group are given in brackets. Results are expressed as means ± S.E.M.

3. Results

3.1. Pretreated animals

Freshly isolated diaphragms

The resting membrane potential of diaphragms freshly isolated from 7-oxo-prostacyclin-pretreated animals was significantly more negative (about 13 mV) than that of diaphragms from non-pretreated controls (Table 1). The effect of 7-oxo-prostacyclin pretreatment on resting membrane potential could be abolished by 1×10^{-4} mol · l^{-1} ouabain. When added to the incubation medium oubain returned the resting membrane potential close to control values (68.30 ± 1.24 mV; $n = 15$) within 5 min.

Na^+-loaded diaphragms

Four hours of loading with sodium decreased the resting membrane potential of diaphragm muscle fibers

Table 1
The effect of 7-oxo-prostacyclin on resting membrane potential in muscle cells from freshly isolated and Na^+-loaded diaphragms

Diaphragm	Resting membrane potential [mV]			
	K^+-free		5 mmol · l^{-1} K^+	
Fresh non-treated			70.78 ± 1.07	(27)
Fresh + pretreatment			83.86 ± 1.40 *	(44)
Na^+-loaded non-treated	61.53 ± 0.84	(41)	79.89 ± 0.98 *	(48)
Na^+-loaded + pretreatment	63.90 ± 1.52	(31)	85.63 ± 2.74 *	(22)

Pretreatment: 7-oxo-prostacyclin was administered at the dose of 50 μg · kg^{-1} i.p. to mice 30–34 h before the experiments. Three muscles were examined and the number of fibers penetrated is given in brackets. Data represent the means ± S.E.M. * Statistically significant changes for $P < 0.05$.

Table 2
Effect of ouabain on Na^+/K^+ pump activation in Na^+-loaded diaphragm muscle fibers: influence of pretreatment with 7-oxo-prostacyclin

Substance	RMP [mV]		Na^+/K^+ pump activation
Na^+-loaded + 10^{-5} M ouabain	64.67 ± 1.66	(17)	–
Na^+-loaded + 10^{-4} M ouabain	64.50 ± 0.81	(43)	–
Pretreatment Na^+-loaded + 10^{-5} M ouabain	70.75 ± 1.39 *	(32)	+
Pretreatment Na^+-loaded + 10^{-4}M ouabain	62.82 ± 1.08	(39)	–

RMP – resting membrane potential measured in Liley solution with 5 mmol · l^{-1} K^+. Pretreatment: 7-oxo-prostacyclin was administered at the dose of 50 $\mu g \cdot kg^{-1}$ i.p. to mice 30–34 h before the experiments. Three muscles were examined and the number of fibers penetrated is given in the brackets. Data represent the means ± S.E.M. * Statistically significant changes for $P < 0.05$.

similarly in both the 7-oxo-prostacyclin-pretreated and in the control groups (Table 1). The presence of 5 mmol · l^{-1} K^+ ions in the incubation medium reversed the effect of Na^+ loading in both groups. However, this reversal was much more pronounced (by approximately 6 mV) in the 7-oxo-prostacyclin-pretreated group. This indicated a more effective involvement of electrogenic Na^+/K^+ pump activation.

In order to check whether the above difference in electrogenicity reflects an enhanced activity of the Na^+/K^+ pump induced by 7-oxo-prostacyclin pretreatment, different concentrations of ouabain were added to the bath. The concentrations of 10^{-4} mol · l^{-1} (n = 43) and 10^{-5} mol · l^{-1} (n = 17) ouabain completely prevented the activating effect of K^+ on the sodium pump in the control diaphragms (Table 2). In diaphragms from 7-oxo-prostacyclin-pretreated mice, however, only the higher 10^{-4} mol · l^{-1} concentration of ouabain (n = 45) was able to abolish the K^+-induced hyperpolarization of the cell membrane (Table 2). Simple washout of ouabain by new Liley solution restored the electrogenic effect in non-pretreated controls, but failed to induce the same effect in 7-oxo-prostacyclin-pretreated muscles.

Ca^{2+} and 7-oxo-prostacyclin pretreatment

An increase in Ca^{2+} concentration in the Liley solution from 2 to 10 mmol · l^{-1} inhibited the electrogenic effect of K^+ ions in Na^+-loaded muscle fibers from the control non-treated group (resting membrane potential = −64.30 ± 1.20 mV; n = 36). The inhibition proved to be reversible during washout. Pretreatment

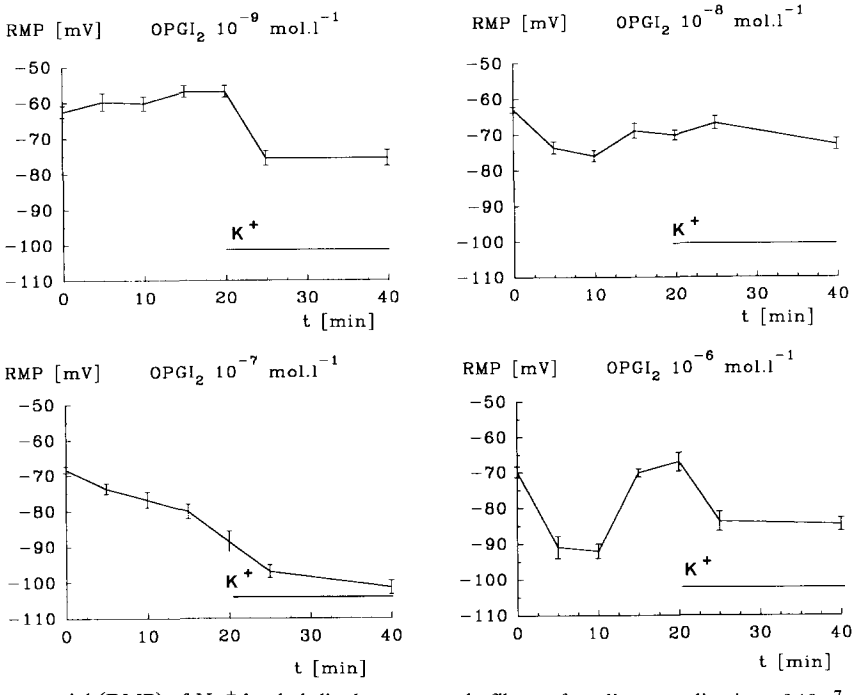

Fig. 1. Resting membrane potential (RMP) of Na^+-loaded diaphragm muscle fibers after direct application of 10^{-7} mol · l^{-1} 7-oxo-prostacyclin and 10^{-4} mol · l^{-1} ouabain (OUA) to the muscle bath. C: RMP values in K^+-free Liley solution (n = 10); 1: RMP values in the presence of 10^{-7} mol · l^{-1} 7-oxo-prostacyclin and 5 mmol · l^{-1} K^+ (n = 10) (indicated below); 2: RMP values in the presence of 10^{-7} mol · l^{-1} 7-oxo-prostacyclin, 5 mmol · l^{-1} K^+ and 10^{-4} mol · l^{-1} ouabain (n = 13). Two numbers of muscles were examined and the number of fibers penetrated is given in brackets. Data represent the means ± S.E.M. Differences with respect to control were considered significant at $P < 0.05$.

with 7-oxo-prostacyclin weakened the effect of the high Ca^{2+} concentration. In this group the K^+-induced hyperpolarization was maintained even in the presence of 10 mmol·l^{-1} Ca^{2+} (resting membrane potential = -77.34 ± 1.61; $n = 38$). Washout of Ca^{2+} ions restored the resting membrane potential to a normal level.

3.2. Acute application of 7-oxo-prostacyclin

Freshly isolated preparation

Five minutes after addition to the bath 7-oxo-prostacyclin (10^{-7} mol·l^{-1}) increased the resting membrane potential of diaphragm muscle fibers from -70.50 ± 0.58 mV ($n = 20$) to -75.30 ± 0.72 mV ($n = 20$). This effect remained stable for a further 5 min.

Na^+-loaded muscles

Addition of 7-oxo-prostacyclin induced a concentration-dependent hyperpolarization of Na^+-loaded diaphragm muscle membranes (Fig. 1). However, the dynamics of this hyperpolarization varied at different concentrations of 7-oxo-prostacyclin with respect to its intensity, initial velocity, and duration. At 7-oxo-prostacyclin concentrations over 10^{-8} mol·l^{-1} the elevation of resting membrane potential could be potentiated by addition of K^+ ions. The maximum hyperpolarization induced by increasing the concentration of 7-oxo-prostacyclin occurred at different times. The data in Fig. 1 revealed no direct relationship between the time of appearance of the peak hyperpolarization and the dose of 7-oxo-prostacyclin.

Direct addition of 7-oxo-prostacyclin to Na^+-loaded diaphragm muscle fibers in Liley K^+ solution (Fig. 2) led to a significant ($P < 0.05$) increase in the resting membrane potential from -65.00 ± 1.09 mV ($n = 10$) to -96.91 ± 1.85 mV ($n = 10$). Subsequent addition of 10^{-4} mol·l^{-1} ouabain to 7-oxo-prostacyclinand 5 mmol·l^{-1} K^+ containing medium decreased the resting membrane potential to -58.46 ± 1.17 mV ($n = 13$).

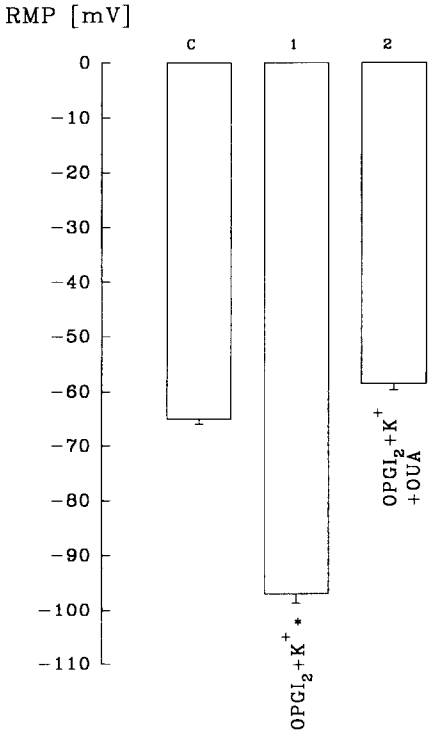

Fig. 2. Time course of changes in the resting membrane potential (RMP) of Na^+-loaded diaphragm muscle fibers after direct application of 7-oxo-prostacyclin ($OPGI_2$) at different concentrations to the muscle bath without and with K^+. 7-Oxo-prostacyclin in concentrations of 10^{-9}–10^{-6} mol·l^{-1}, K^+ addition of 5 mmol·l^{-1} K^+. Data are means ± S.E.M. from 10–30 separate measurements.

4. Discussion

The anti-arrhythmic and anti-ischemic effects of 7-oxo-prostacyclin on the heart have been extensively investigated and documented (Udvary and Szekeres, 1986). They are ascribed in part to: (a) prolongation of the action potential and of the effective refractory period (Szekeres et al., 1983, 1987); and (b) a positive influence of 7-oxo-prostacyclin on the integrity of cardiac cell membranes (Szekeres et al., 1989). Nevertheless, there are still no data concerning other types of muscles.

In the present study we investigated the effect of 7-oxo-prostacyclin on membrane function of skeletal muscle cells. The late effect of 7-oxo-prostacyclin achieved by pretreatment with this substance involved an increase in the resting membrane potential in fresh muscle preparations and the enhancement of the electrogenic effect in Na^+-loaded muscles evoked by addition of external K^+. Both these effects were sensitive to ouabain.

The difference in resting membrane potential values of Na^+-loaded cells from non-treated or 7-oxo-prostacyclin-treated tissues may be explained by: (i) a direct action of 7-oxo-prostacyclin on sodium pump activity as reflected by an increased Na^+/K^+-ATPase activity; (ii) an indirect action of 7-oxo-prostacyclin involving an increase in Na^+ permeability of 7-oxo-prostacyclin-treated muscle, which leads to higher Na^+ loading of the cells and a higher pumping activity triggered by elevated intracellular Na^+ ions.

It has been shown that it takes several hours for the full effect of 7-oxo-prostacyclin to develop, the active principle of which is the modulation of heart sarcolemmal Na^+/K^+-ATPase (Džurba et al., 1991). These authors observed an almost 2-fold increase in sarcolemmal Na^+/K^+-ATPase activity in rats pretreated

with 7-oxo-prostacyclin (50 $\mu g \cdot kg^{-1}$) for 1–2 days. This increase in enzyme activity was explained by an increased synthesis of new molecules of Na^+/K^+-ATPase with increased affinity for ATP, because simultaneous i.m. administration of the proteosynthesis blocking drug, cycloheximide, abolished the effect of 7-oxo-prostacyclin pretreatment substantially. The synthesis of additional new molecules of Na^+/K^+-ATPase is probably responsible for the observed increase in activity of the electrogenic sodium pump and the coupled elevation of the resting membrane potential value. A similar mechanism may also be responsible for the late effect of 7-oxo-prostacyclin on the sodium pump in skeletal muscle. It may also explain the lowered sensitivity of the Na^+/K^+ pump to the influence of external Ca^{2+} or ouabain.

The acute immediate effect of 7-oxo-prostacyclin may have a different molecular mechanism. One possibility seems to be the temporary opening of additional membrane K^+ channels. This speculation is based on the findings that arachidonic acid and its metabolites activate the electrogenic Na^+/K^+-ATPase in muscle (Vyskočil et al., 1987) as well as the K^+ channel in atrial myocytes (Kurachi et al., 1989).

Although the mechanisms for the immediate and late effects of 7-oxo-prostacyclin may be different, our results indicate that the action of this substance involves modulation of the resting membrane potential. This modulation evidently occurs via the action of 7-oxo-prostacyclin on the electrogenic Na^+/K^+ pump. The potentiation of cell electrogenesis described here can therefore be added to the list of 7-oxo-prostacyclin actions.

Acknowledgements

This work was supported, in part, by the Slovak Grant Agency for Science (grant No. 750/93) and by the Grant Agency for Science of the Czech Republic No. 204/93/0704. The authors are particularly indebted to Prof. Dr. L. Szekeres from the Institute of Pharmacology, Albert Szent-Gyorgyi University of Szeged (Hungary) for the kind gift of the new substance (7-oxo-prostacyclin) as well as for valuable advice. The authors wish to express their thanks to Dr. J. Slezák, DrSc., Director of the Institute for Heart Research, for supporting this research project and to Dr. Ruth Payne for her helpful comments.

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