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Effects of 3,4-diaminopyridine on myoplasmic calcium and phosphoinositide hydrolysis in frog sartorius muscle fibers[1]

XU Ze-Min, DONG Zheng, ZHU Pei-Hong
(Shanghai Institute of Physiology, Chinese Academy of Sciences, Shanghai 200031, China)

ABSTRACT Using Ca^{2+}-selective microelectrodes, the effects of 3, 4-diaminopyridine (DAP) on myoplasmic calcium ($[Ca^{2+}]_i$) were studied in frog satorius muscle fibers. DAP induced an increase of $[Ca^{2+}]_i$ in a concentration-dependent manner. DAP 1 mmol·L^{-1} increased the $[Ca^{2+}]_i$ from control 166±41 nmol·L^{-1} to 416±69 nmol·L^{-1} ($n=10$). In the absence of external calcium, DAP still enhanced $[Ca^{2+}]_i$. $[Ca^{2+}]_i$ of the fibers bathed in Ca^{2+}-free Ringer's solution containing DAP 1 mmol·L^{-1} was 152±43 nmol·L^{-1}, which was significantly higher than 77±35 nmol·L^{-1} of $[Ca^{2+}]_i$ of the fibers in Ca^{2+}-free Ringer's solution. In addition, DAP promoted the hydrolysis of phosphoinositides, and DAP-induced hydrolysis was more in the presence of external calcium. It is suggested that, through enhancing the hydrolysis of phosphoinositides, DAP released Ca^{2+} from intracellular Ca^{2+} store in frog sartorius muscle fibers.

KEY WORDS aminopyridines; cytosol; calcium; phosphoinositides; muscles

3,4-Diaminopyridine (DAP), known as a K^+ channel blocker, produced asynchronous oscillations of myoplasmic calcium ($[Ca^{2+}]_i$) and contractures of frog twitch muscle fibers[1]. These effects of DAP appeared also in Ca^{2+}-free medium, suggesting that DAP released Ca^{2+} from intracellular Ca^{2+} store by an unclear mechanism[1]. Inositol 1,4,5-trisphosphate, a product of phosphatidylinositol 4,5-bisphosphate hydrolysis, has such a function in various cells[2-4]. The present study aims at exploring whether or not DAP, aside from blocking some types of K^+ channels, can release Ca^{2+} from intracellular Ca^{2+} store through enhancing the hydrolysis of phosphoinositides.

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MATERIALS AND METHODS

Chemicals and preparations DAP was purchased from Aldrich, and Calcium-cocktail for making Ca^{2+}-selective microelectrodes was obtained from Fluka. [^{3}H]Myo-inositol (specific activity 518 TBq·mol^{-1}) was provided by Shanghai Institute of Nuclear Research, Chinese Academy of Sciences. Dowex AGI-X8 (anion exchange resin) was purchased from Bio-Rad. The composition of Ringer's solution was NaCl 120, KCl 2, $CaCl_2$ 1.8, and HEPES 10 mmol·L^{-1}, while Ca^{2+}-free Ringer's solution consisted of NaCl 120, KCl 2, $MgCl_2$ 5, egtazic acid (EGTA) 1, and HEPES 10 mmol·L^{-1}.

The solutions were titrated to pH 7.2. The experiments were performed on the isolated sartorius muscles of *Rana nigromaculata* at 15±1 C.

$[Ca^{2+}]_i$ measurement Under a light microscope (Opton, X100), a reference microelectrode filled with KCl 3 mol·L^{-1} and a calibrated Ca^{2+}-selective microelectrode were impaled into one fiber, and the potentials of the microelectrodes were monitored by an electrometer FD-223 (World Precision Instruments). After each insertion, the Ca^{2+}-selective microelectrode was calibrated again, and the value of $[Ca^{2+}]_i$ was read from the calibration curve[5].

Measurement of phosphoinositides hydrolysis[6] A pair of muscles was incubated in Ringer's solution containing [^{3}H]Myo-inositol 0.7 μmol·L^{-1} for 12 h, and LiCl 20 mmol·L^{-1} was supplemented to the medium in the last hour. After labeling, one preparation was treated with Ringer's solution containing DAP 1 mmol·L^{-1} for 30 min, and the other was bathed in Ringer's solution as control. Then, the preparation was immediately put onto a liquid N_2-cooled copper

plate, smashed and ground into fine powder in liquid N_2. Inositol phosphates were extracted with trichloroacetic acid 0.92 mol·L^{-1} and fractionalized by anion exchange chromatograghy. The column was eluted with ammonium formate 1 mol·L^{-1}-formic acid 0.1 mol·L^{-1} to obtain the total inositol phosphates (IP), which was counted by a liquid scintillator.

RESULTS

Effect of DAP on $[Ca^{2+}]_i$ $[Ca^{2+}]_i$ of the fibers in Ringer's solution was first measured as control, and then the effect of DAP on $[Ca^{2+}]_i$ was examined 15 min after adding DAP into the medium. The measurement usually took 4—5 h, and no change of $[Ca^{2+}]_i$ was noticed within the bathing time. $[Ca^{2+}]_i$ increased with raising DAP concentration, and reached a peak at DAP 1 mmol·L^{-1}. $[Ca^{2+}]_i$ declined with the further increase of DAP concentration (Fig 1).

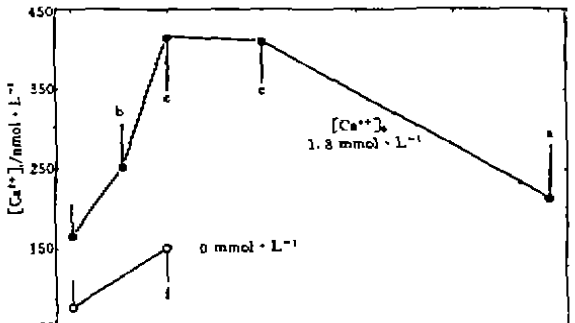

Fig 1. Effect of DAP on myoplasmic calcium of frog sartorius muscle fibers. $n = 9 - 14$ fibres. $\bar{x} \pm s$, $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$, and $^dP < 0.01$ vs $[Ca^{2+}]_i$ at DAP 0 mmol·L^{-1} in either $[Ca^{2+}]_o$ 0 mmol·L^{-1}(○) or 1.8 mmol·L^{-1}(●), respectively.

To analyze the source of Ca^{2+} in DAP-induced increase of $[Ca^{2+}]_i$, the effects of DAP 1 mmol·L^{-1} on the $[Ca^{2+}]_i$ in the presence and absence of external calcium were compared. In its presence, the $[Ca^{2+}]_i$ increased from 166 ± 41 nmol·L^{-1} to 416 ± 69 nmol·L^{-1}. Bathing the fibers in Ca^{2+} free Ringer's solution itself reduced the $[Ca^{2+}]_i$ to 77 ± 35 nmol·L^{-1}. DAP resulted a
crease of the $[Ca^{2+}]_i$ in Ca^{2+}-free m
the extent of the increase was ob
than that in the presence of exte
(Fig 1).

A hyperpolarization of the re
brane potentials was noted in the fi
in DAP containing Ringer's solut
did not produce such a hyperpol
Ca^{2+}-free medium (Tab 1).

**Tab 1. Effects of DAP on the resting m
tential (Em) in the presence and absen
calcium. $\bar{x} \pm s$. $^aP > 0.05$ vs Em at DA
in the absence of external calcium. $^bP <$
DAP 0 mmol·L^{-1} in the presence of exte**

$[Ca^{2+}]_o$/ mmol·L^{-1}	DAP/ mmol·L^{-1}	Fiber number	
0	0	10	—
	1	10	—
1.8	0	10	—
	0.5	9	—1
	1	10	—1
	5	14	—1

**Effects of DAP on hydrolysis
inositides** In the presence of ex
um, exposing the preparation to D
· L^{-1} for 3 0 min increased the
g·muscle) from 13692 ± 1887 to 17
(4 pairs of preparations), indicat
hydrolysis of phosphoinositides, w
by DAP. In Ca^{2+}-free medium
g·muscle) increased from 13788
16 443 ± 1 951 (4 pairs of prepa
such a treatment (Tab 2).

DISCUSSION

Our results clearly indicated
could increase the $[Ca^{2+}]_i$ and enh
drolysis of phosphoinositides in f
muscle. Since DAP-induced o
myoplasmic Ca^{2+} was monitored

Tab 2. Effect of DAP (1 mmol·L^{-1}, 30 min) on total inositol phosphates (IP) in frog sartorius muscle in the presence and absence of external calcium. $n = 4$, $\bar{x} \pm s$. $^{c}P < 0.01$ *vs* control (100 %) in $[Ca^{2+}]_o$ 0 mmol·L^{-1}. $^{b}P < 0.05$ *vs* control (100 %) in $[Ca^{2+}]_o$ 1.8 mmol·L^{-1}.

$[Ca^{2+}]_o$, mmol·L^{-1}	IP, dpm/g muscle Control	DAP	Ratios, %
0	13 788±1 855	16 443±1 951	120±3^{c}
1.8	13 692±1 887	17 595±1 280	130±5^{c}

III[13], it is impossible to know the exact value of $[Ca^{2+}]_i$ prior to this study. This is also the first report on the effect of DAP on the hydrolysis of phosphoinositides in skeletal muscle.

Because the effects of DAP on the $[Ca^{2+}]_i$ were still present in Ca^{2+}-free medium, it is implied that DAP could release Ca^{2+} from intracellular Ca^{2+} store. At present, it can not be excluded that DAP might have a direct effect on intracellular Ca^{2+} store. But, according to our results just mentioned and the well known function of inositol 1, 4, 5-trisphophate[14], we prefer that DAP might release Ca^{2+} from the intracellular Ca^{2+} store through enhancing the hydrolysis of phosphoinositides. This proposal is also supported by the fact that the removal of external calcium caused parallel changes of phosphoinositide hydrolysis and $[Ca^{2+}]_i$. We have not investigated how the effect on phosphoinositide hydrolysis depends on the DAP concentration in skeletal muscle, but the dose dependence has been observed on the cultured neurons of embryo chicken fore-brain. It was found that a maximum effect on the hydrolysis of phosphoinositides was also seen at DAP 1 mmol·L^{-1} (unpublished data). On the whole, it is suggested that there exists a causal relationship between the hydrolysis of phosphoinositides and the increase of $[Ca^{2+}]_i$ brought by DAP.

As the Ca^{2+}-dependent K^+ channels are present on the membrane of skeletal muscle fibers[17], a hyperpolarization can be expected if $[Ca^{2+}]_i$ would be enhanced to a certain level. In Ca^{2+}-free medium DAP caused an increase of $[Ca^{2+}]_i$, but $[Ca^{2+}]_i$ in this case was still slightly lower than $[Ca^{2+}]_i$ of the fibers in Ringer's solution. This may account for that the DAP-induced hyperpolarization was only noticeable in the presence of external calcium.

REFERENCES

1 Miledi R, Parker I, Zhu PH. Extracellular ions and excitation-contraction coupling in frog twitch muscle fibres. J Physiol (Lond) 1984; **351**: 687—710.
2 Berridge MJ, Irvine RF. Inositol phosphates and cell signalling. Nature 1989; **341**: 197—205.
3 Hidalgo C, Jaimovich E. Inositol trisphosphate and excitation-contraction coupling in skeletal muscle. J Bioenerg Biomembr 1989; **21**: 267—87.
4 Irvine RF. Inositol 1,4,5-trisphosphate and inositol 1,3,4,5-tetrakisphosphate: two second messengers in a duet of calcium regulation. In: Vanderhoek JY, editor. Biology of cellular transducing signals. New York: Plenum Press, 1990; 213—25.
5 Zhu PH, Sun YB. Measurement of the myoplasmic free Ca^{2+} concentration of frog twitch muscle fibres in high K^+ solution. Chin J Physiol Sci 1987; **3**: 384—89.
6 Dong Z, Chen KY, Zhu PH. Effects of high K^+ exposure on phosphoinositide metabolism in frog skeletal muscle. Biochem Biophys Res Commun 1992; **182**: 767—72.
7 Blatz AL, Magleby KL. Calcium activated potassium channels. Trends Neurosci 1987; **10**: 463—67.

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3,4-二氨基吡啶对蛙缝匠肌纤维肌浆钙离子和肌醇磷脂水解的影响

徐泽敏，董 政，朱培闻
(中国科学院上海生理研究所，上海200031，中国)

摘要 3,4-二氨基吡啶(DAP)可以浓度依赖性地增加$[Ca^{2+}]_i$. DAP 1 mmol·L^{-1}使$[Ca^{2+}]_i$由对照的166±41 nmol·L^{-1}升高到416±69 nmol·L^{-1}($n=10$). 在胞外无钙时，DAP仍使$[Ca^{2+}]_i$升高. DAP还促进肌醇磷脂水解. 在有钙溶液中，DAP引起的肌醇磷脂水解更多. 结果提示，DAP可能通过促进蛙骨骼肌纤维的肌醇磷脂水解，使细胞内钙库释放钙.

关键词 氨基吡啶类；胞浆；钙；肌醇磷脂；肌肉