Short communication

Effects of Dihydropyridine CGP on Currents through the Calcium Channels in Frog Skeletal Muscle

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CGP (2-methyl-3-methoxycarbonyl-4-(2′-difluoromethoxyphenyl)-5-oxo-1,4,5,7--tetra-hydrofuro-(3,4-b)pyridine) is thought to be a calcium channel agonist, like CGP 28392 (Fossheim 1987) which produces agonist response probably due to the lactose ring forcing the carbonyl group to adopt an antiplanar conformation. The aim of this study was to investigate the action of CGP on currents through the slow Ca channels in skeletal muscle during depolarizing impulses and at rest. The protein components of the slow calcium channels are known to posses binding sites for dihydropyridine (DHP) (for a review see Glossmann et al. 1988).

In our experiments transmembrane Ba^{2+} currents through calcium channels were recorded in isolated twitch muscle fibre segments from m. ileofibularis of *Rana temporaria* by means of the vaseline-gap voltage clamp method (Zachar et al. 1982) at 18—20 °C. High Ba^{2+} solution (in mmol/l: $BaCl_2$ 10; TEABr 99; KCl 2.5; TRIS-HCl 10; glucose 5.6; TTX 10^{-7} mol/l) was used as the external solution. Both ends of the fibre segments were immersed in internal solution containing (in mmol/l): TEABr 60; $CsCl_2$ 50; EGTA 0.1; TRIS-HCl 10. pH of both solutions was 7.5. CGP was dissolved in dimethylsulphoxide and then added to the external solution. The final CGP concentration was 5×10^{-5} mol/l.

Fig. 1*A* shows the action of CGP on I_{Ba} recorded in muscle fibre segments under voltage clamp conditions. The agonist effect of this substance on Ca channels is obvious. Maximum I_{Ba} increased by 40 % (35 % ± 3 %, $n = 7$); the tail current also essentially increased and was prolonged (Fig. 1*C*). The threshold potential for activation of I_{Ba} was reduced by 20 mV (Fig. 1*B*). Similar effects on Ca channels in mammalian skeletal muscle were reported recently for DHP derivative Bay K 8644 (Cognard et al. 1986; Lamb and Walsh 1987).

Next, the effects of CGP on Ca^{2+} channels in whole resting muscle were investigated. The technique using Sr^{2+} to extract the rapidly exchanging Ca^{2+} pool was employed (Bianchi and Narayan 1982). Sr^{2+}, Ca^{2+} and Mg^{2+} con-

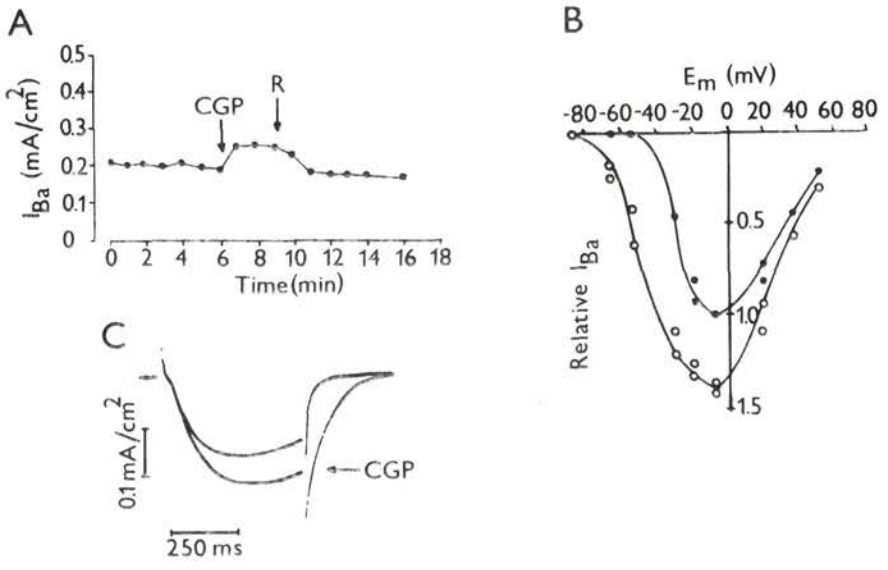

Fig. 1. *A* — CGP (5×10^{-5} mol/l)-induced increase of I_{Ba}. The second arrow indicates wash-out of the drug. *B* — I—V characteristics of the muscle membrane for Ba currents in normal solution (filled symbols) and in the presence of CGP (same concentration, open circles). Note the higher current amplitudes and the lower threshold in the presence of the drug. *C* — Ba currents in normal solution (upper trace) and in solution with 5×10^{-5} mol/l CGP (lower trace). Holding potential — 100 mv throughout. In *B* and *C*, CGP was present in the solution for 5 min.

centrations were determined in paired sartorius and ileofibularis muscles isolated from *Rana temporaria*. After dissection the muscles were equilibrated in Ringer solution (R) (in mmol/l: NaCl 111; KCl 2.5; $CaCl_2$ 1.8; TRIS-HCl buffer, pH 7) for 60 min. Subsequently, the control muscles were exposed to Sr^{2+}-Ringer solution (in mmol/l: NaCl 111; KCl 2.5; $SrCl_2$ 1.5; TRIS-HCl, buffer, pH 7) for 20—60 min and the corresponding paired muscles to Sr^{2+}-Ringer containing 2×10^{-4} mol/l CGP for the same time period. The muscles were then extracted overnight in 4 ml of 0.1 N HCl. Sr^{2+}, Ca^{2+} and Mg^{2+} in the solution were determined with a Perkin Elmer flame photometer. At the end of the experiment the muscles were dried and weighed. The cation contents were expressed as μmol/g dry weight.

Table 1 summarizes the effects of CGP (2×10^{-4} mol/l) on the uptake of divalent cations in resting muscle. CGP significantly increased the Sr^{2+} (by 20 %) and Ca^{2+} contents (by 13 %), whereas Mg^{2+} content remained unaffected. Since Sr^{2+} ions pass well through the Ca^{2+} channels (Almers and Palade 1981), the increased muscle content of Sr^{2+} can be the result of a higher probability of the open state and a prolonged mean open time of these channels due to the

Table I. The effects of the dihydropyridine derivative CGP on Sr^{2+}, Ca^{2+} and Mg^{2+} contents in frog muscles

No	Sr^{2+} (μmol/g dr. w.)			Ca^{2+} (μmol/g dr. w.)			Mg^{2+} (μmol/g dr. w.)		
	SrR	SrR + CGP	Change (%)	SrR	SrR + CGP	Change (%)	SrR	SrR + CGP	Change (%)
1 (i)	2.20	2.48	+12.7	6.9	7.2	+4.3			
2 (s)	0.99	1.03	+4.0	6.8	9.9	+45.6			
3 (i)	1.51	1.55	+2.6	9.4	11.2	+19.1			
4 (i)	0.98	1.34	+36.7	6.8	8.0	+17.6			
5 (i)	1.14	1.25	+9.6	5.6	5.6	0.0			
6 (s)	2.89	4.16	+43.9	17.2	15.0	−12.8	49.83	50.20	+0.7
7 (s)	2.78	3.23	+16.2	12.2	13.8	+13.1	48.17	49.25	+2.2
8 (s)	2.39	3.36	+40.6	3.9	4.9	+25.6	23.18	23.46	+1.2
9 (s)	3.54	3.97	+12.1	5.6	5.9	+5.4	43.46	40.77	−5.9
Mean ± S.E.M.			19.8 ± 5.4 $p < 0.01$			13.1 ± 5.6 $p < 0.05$			−0.45 ± 1.8 $p > 0.10$

(i) — m. ileofibularis; (s) — m. sartorius; change (%) — differences in the cation content between SrR + CGP and SrR muscles expressed in % of that in SrR muscle. Muscles 1—5 were washed with Ringer solution for 4—7 min; muscles 6—9 were washed with isotonic sucrose solution for 4 s.

effect of CGP. Thus, the increase in cell Sr^{2+} probably reflects an increased Sr^{2+} influx. The CGP-induced increase in Ca^{2+} content can be interpreted as being the result of competitive inhibition by Sr^{2+} of Ca^{2+} efflux via the Na-Ca^{2+} exchange mechanism.

Following conclusions can be drawn based on the results obtained: 1) CGP acts as an agonist on Ca^{2+} channels in frog skeletal muscle fibres and whole resting muscles; 2) although the muscle Sr^{2+} content reflects the rapidly exchanging Ca^{2+} component localized mostly in the extracellular water space and bound to connective tissue and muscle fiber surface sites, it might be suggested that a portion of rapidly exchanging Ca^{2+}, namely the CGP-induced increment, is localized inside the muscle fibres.

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References

Almers W., Palade P. T. (1981): Slow calcium and potassium current across frog muscle membranes: measurements with a vaseline-gap technique. J. Physiol. (London) **312,** 159—176

Bianchi C. P., Narayan S. (1982): Possible role of transverse tubules in accumulating calcium released from the terminal cisternae by stimulation and drugs. Can. J. Physiol. Pharmacol. **60,** 503—507

Cognard C., Romey G., Galizzi J. P., Fosset M., Lazdunski M. (1986): Dihydropyridine-sensitive Ca^{2+} channels in mammalian skeletal muscle cells in culture: electrophysiological properties and interactions with Ca^{2+} channel activator (Bay K8644) and inhibitor (PN 200-110). Proc. Nat. Acad. Sci. USA **83,** 1518—1522

Fossheim R. (1981): Crystal structure of a 1.4-dihydropyridine with enantiomers showing opposite effects on calcium channel agonists and antagonists. Acta Chem. Scand., Ser. B **41,** 581—588

Glossmann H., Striessing I., Terry D. K., Goll A., Moosburger K., Schirmer M. (1988): Interaction between calcium channel ligands and calcium channels. Circ. Res. **61,** Suppl. I, 30—36

Lamb G. D., Walsh T. (1987): Calcium currents, charge movement and dihydropyridine binding in fast- and slow-twitch muscles of rat and rabbit. J. Physiol. (London) **393,** 595—617

Zachar J., Zacharová D., Henček M., Nasledov G. A., Hladký M. (1982): Voltage-clamp experiments in denervated frog tonic muscle fibres. Gen. Physiol. Biophys. **1,** 385—402

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