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COMMENTARY

Statins and fibrate target ClC-1 – from side effects to CLC pharmacology

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Lipid-lowering drugs have been associated with severe adverse effects on skeletal muscle, including rhabdomyolysis. More common symptoms include cramps and myalgia. In this issue of the *BJP,* Pierno *et al.* find that two statins and fenofibrate, often used in combination, decrease function of the chloride channel member 1 of the mammalian CLC protein family of chloride channels and transporters in skeletal muscle by three distinct mechanisms. Other compounds have recently been shown to potently modulate the pharmacologically less well-explored CLC protein family, which comprises chloride channels and chloride/proton antiporters. These findings may well lead to a new era of CLC protein pharmacology.
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Abbreviations: 9-AC, 9-anthracene carboxylic acid; β-HMG-CoA, β-hydroxymethyl-glutaryl-coenzyme A; ClC-1, member 1 of the mammalian CLC protein family of chloride channels and transporters; DIDS, 4,4′-diisothiocyano stilbene-2,2′-disulphonic acid; PKC, protein kinase C

Hypolipidemic drugs are widely used to lower cholesterol in patients who suffered from a heart attack or stroke, or exhibit elevated cardiovascular risk factors like hypertension and high low-density lipoprotein cholesterol. In an ageing society, there may be good reasons to put a majority of patients on one of these drugs, with the β-hydroxymethyl-glutaryl-coenzyme A(β-HMG-CoA) reductase inhibitors, the statins, being the most common, widely used and most effective agents. These have also be shown to be beneficial in Alzheimer's disease (Jick *et al.*, 2000), an effect which may be unrelated to their hypolipidemic action, and this may well broaden their use further in the future. However, as for most effective drugs, there is no beneficial action without adverse effects. Some of these are associated with their lipid-lowering action itself, but others may be due to off-target effects. Adverse effects involving skeletal muscle are particularly common and may be life-threatening in rare instances. In fact, a recently introduced β-HMG-CoA reductase inhibitor, cerivastatin, had to be withdrawn from the market because of an elevated incidence of rhabdomyolysis, the most severe condition encountered with the use of these drugs. Concomitant use of statins and fibrates with the aim of improving lipid profiles further increase the incidence of this complication. Its manifestation may be related to the plasma level of the drugs, as it is more common in patients treated with higher doses, or on other drugs that increase the plasma levels of statins or fibrates. There is evidence that sequence variations in an organic anion transporter involved in hepatic uptake of statins may affect strongly the risk to suffer from muscle side effects (Link *et al.*, 2008), which also include cramps and myalgia upon exercise.

In the current issue of *BJP*, Pierno *et al.* (2009) provide experimental evidence, extending previous studies from the same laboratory and others, which will help to understand most, if not all, of the impact lipid-lowering agents may have on skeletal muscle. Pierno *et al.* investigate the effect of the two most commonly used hypolipidemic drugs classes, statins and fenofibrate, on the major skeletal muscle chloride channel, member 1 of the mammalian CLC protein family of chloride channels and transporters (ClC-1). This channel controls muscle excitability by stabilizing the membrane voltage of the skeletal muscle cells, preventing repetitive runs of discharges following an action potential. These discharges are known as myotonic runs and can be found in humans and animals with mutations in ClC-1 channels (Steinmeyer *et al.*, 1991; Koch *et al.*, 1992). Mutations can be both dominant and recessive, with the former ones usually affecting the voltage dependence of the slow gate of ClC-1 channels, preventing activation of ClC-1 channels at physiological membrane voltages (Pusch *et al.*, 1995). A chloride conductance, rather than the more

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commonly used potassium channels, has evolved to stabilize muscle membrane voltage probably because potassium concentrations in the small intercellular clefts between muscle cells would rise from the physiological 4 $mmol \cdot L^{-1}$ to levels significantly depolarizing the membrane during repetitive discharges. In contrast, a change of the extracellular chloride concentration (in excess of 100 $mmol \cdot L^{-1}$ under physiological conditions) by a few $mmol \cdot L^{-1}$ will hardly change the chloride equilibrium potential significantly. Intracellular chloride concentrations will remain sufficiently stable due to the larger intracellular volume.

ClC-1 channels can be blocked, relatively specifically, by 9-anthracene carboxylic acid (9-AC) and the authors use this compound to compare the effects of inhibiting ClC-1 channels with the effects of hypolipidemic drugs. Interestingly, myotonic discharges resembling those induced by acute 9-AC treatment in experimental animals did not develop in rats acutely treated with any of the lipid-lowering drugs, but only after treatment for a few weeks. They identify three different modes by which these drugs affect ClC-1 channel function. It has been shown that activity of the CLC-1 channel is regulated by phosphorylation (Rosenbohm *et al.*, 1999). Protein kinase C (PKC), activated by lipids and calcium, decreases the activity of ClC-1 channels. By restoring ClC-1 channel activity *in vitro* with chelerythrine, a PKC inhibitor, they show that different statins, to a different degree, activate PKC, possibly by releasing calcium from mitochondria. Fenofibrate, in contrast, directly inhibits ClC-1 channel activity. However, the most important mechanism may be a striking (by a factor of up to 30) down-regulation of ClC-1 channel mRNA by both classes of drugs. Slowly decreasing expression of ClC-1 channels may also explain why myotonic-like discharges can only be observed after chronic administration. This finding is surprising, and warrants further studies into its mechanism, which may well help to understand regulation of ClC-1 channel expression under physiological conditions.

Direct action on ClC-1 channels may, if it extends to other CLC proteins, provide a good starting point to find drugs that modulate this so far pharmacologically poorly explored class of proteins. Many of them are widely expressed in a variety of mammalian cell types, making them less attractive targets, but some, like ClC-1, ClC-Ka, ClC-Kb and ClC-6, are confined to very specific structures. Obviously, activators for ClC-1 channels would be beneficial in myotonia patients. That an era of CLC pharmacology has begun, is suggested by the recent finding that certain derivatives of 4,4′-diisothiocyano stilbene-2,2′-disulphonic acid (DIDS) potently inhibit several different CLC proteins (Matulef *et al.*, 2008). Mammalian CLC proteins may be channels or chloride/proton antiporters (Picollo and Pusch, 2005; Scheel *et al.*, 2005), and so far specific drugs have only been identified that act on the channel branch of this family. However, these new DIDS derivatives also inhibit the bacterial analogue *Escherichia coli* CLC protein 1, an established transporter, and may therefore target a more conserved structure than 9-AC, which is specific for ClC-1. Another class of drugs, derivatives of flufenamic acid, is probably lacking activity on transporter type CLC proteins, but can potentiate, as well as inhibit, ClC-K channels. Major structural determinants for this differential action have been elucidated (Liantonio *et al.*, 2008). Therefore, the future may not look entirely dim for CLC protein pharmacology!

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