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An ion channel hypothesis to explain divergent cardiovascular safety of COX-2 inhibitors: the answer to a hotly-debated puzzle?

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Running title: Ion channels explain divergent CV safety of COX-2 inhibitors.

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Abstract.

Cyclooxygenase inhibitors represented extremely promising novel anti-inflammatory drugs until one of them, rofecoxib (Vioxx®), was found to be associated with increased cardiovascular morbidity; however, another such drug, celecoxib (Celebrex®) suffers far less from this side-effect for unknown reasons and is still widely used. This *Perspective* comments on the paper by Bruggemann et al. in this issue that suggests a hypothesis. Celecoxib, but not rofecoxib, is shown to act as an "opener" of voltage-gated KCNQ5 K^+ channels, and a blocker of "L-type" Ca^{2+} channels, causing a reduction in the excitability and contractility of vascular smooth muscle cells (VSMCs). Furthermore, VSMC tone is shown to be selectively reduced by celecoxib, resulting in dilation of blood vessels and reduction in systemic blood pressure, suggesting that the reduced work load on the heart may counteract any other deleterious effects of this class of drugs. This *Perspective* discusses these findings in light of the role of KCNQ K^+ channels in control of excitability in general, the "lipid imbalance theory" of COX-2 risks, and the potential for novel therapeutic modalities for cardiovascular disease focused on ion channels in vascular smooth muscle.

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The development of non-steroidal anti-inflammatory drugs (NSAIDs) selective for the cyclo-oxygenase-2 (COX-2) enzyme ten years ago appeared to be a major breakthrough for the amelioration of a variety of inflammatory disease states. Although the traditional NSAIDs are equally efficacious against inflammatory processes, they also suppress the COX-1 enzyme responsible for cytoprotective actions in the gastrointestinal system, making them ulcerogenic for many people. The two most popular COX-2 selective drugs (COXIBs) were rofecoxib (Vioxx®) and celecoxib (Celebrex®), which garnered sales of $1.5 billion and $400 million, respectively, in their first year alone. Moreover, the COX-2 enzyme was shown to be heavily used by cancerous colonic cells, raising the prospect that the COXIBs could work as preventative anti-cancer drugs as well. However, trouble loomed for the COXIB success story. In the rofecoxib colon cancer prevention trial (APPROVe), whereas the patients on the drug experienced a 24% reduction in the recurrence of colonic polyps, they also suffered a 1.92-fold increase in cardiovascular (CV) side effects – mostly heart attacks and strokes (Bresalier et al., 2005). Within days of reports of these CV side-effects of rofecoxib, Merck withdrew Vioxx® from the market, and has been subject to much litigation from patients taking the drug who experienced such CV events. Likewise, another selective COX-2 inhibitor, valdecoxib, suffers from the same problems (Nussmeier et al., 2005). On the other hand, the CV risk profile of celecoxib is much lower, with most studies suggesting the risk to be only minor (Dajani and Islam, 2008; McGettigan and Henry, 2006). What is the reason for this seeming discrepancy among COX-2 inhibitor drugs in their adverse CV effects?

In this issue of *Molecular Pharmacology*, Brueggemann et al. provide a hypothesis based on drug-selective actions on two critical ion channels in vascular smooth muscle cells (VSMCs). The channels are voltage-gated K^+ channels of the KCNQ (Kv7, "M-type") class, and "L-type" voltage-gated Ca^{2+} channels (VGCCs). Like all smooth muscle, the tone of VSMCs depends on intracellular [Ca^{2+}], which is largely determined by the opening of VGCCs, which in turn is determined by membrane potential. M-type K^+ channels, so named for their suppression by muscarinic agonists in nerve (Constanti and Brown, 1981), are expressed in a variety of

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excitable cells where they play a dominant role in regulation of the resting potential and of action potential firing (Brown, 2008). Thus, suppression of M currents in the nervous system by neurotransmitters and hormones results in increased neuronal firing, inherited mutations of KCNQ2 and KCNQ3 genes cause epileptic syndromes in human infants due to neuronal hyper-excitability, and dysfunctional KCNQ1-containing channels in the heart are responsible for cardiac arrhythmias (Hernandez et al., 2008; Maljevic et al., 2008; Peroz et al., 2008). With the discovery by several labs of several KCNQ subtypes in smooth muscle arose the possibility of the control of vascular tone by M-channel activity (Mackie and Byron, 2008). Indeed, KCNQ1, KCNQ4 and KCNQ5 have been identified in VSMCs, and KCNQ5 activity appears instrumental to the vasoconstrictor response to the hormone arginine vasopressin (AVP) (Mackie et al., 2008; Yeung et al., 2007). How does this fit in with the COXIB story? In a stroke of breathtaking serendipity, Brueggemann et al. here show that celecoxib, but not rofecoxib nor diclofenac (another COXIB), acts as a KCNQ channel opener. Moreover, only celecoxib reversed AVP-induced Ca^{2+} spiking and vasoconstriction. Thus, celecoxib would act in smooth muscle similar to other KCNQ openers (retigabine, flupirtine, meclofenamic acid) (Yeung et al., 2007), resulting in a hyperpolarization of the resting potential, vasodilation and a brake on vasopressin-induced constriction. Indeed, celecoxib seems to act similarly in VSMCs as do neuronal M-channel openers: the reduction of excitability and a brake on action potentials (Gamper et al., 2006; Lerche et al., 2001).

However, we must not forget about the 2^{nd}-part of this two-part discovery. Indeed, Brueggemann et al. also show that celecoxib, but again not rofecoxib nor diclofenac, are potent inhibitors of L-type VGCCs. Thus, not only does celecoxib open the K^+ channels that retard depolarizations and action potentials, but it also inhibits the Ca^{2+} channels whose activity is required for rises in intracellular Ca^{2+}, excitation/contraction coupling and vasoconstriction. The end result of this *one-two* punch is celecoxib-mediated relaxation of the VSMCs, vasodilation, and presumably a reduction in systemic blood pressure. The relationship between systemic blood pressure and myocardial infarction is well known, with the CV risk doubling for each increment of 20/10 mmHg of the systolic/diastolic pressures above 115/75 mmHg (Chobanian et al., 2003). The effect of celecoxib

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in blockade of AVP actions in mesenteric artery shown in Brueggemann et al. is particularly interesting in light of the heavy association of cerebral vasospasm with high AVP levels in the brain (Delgado et al., 1988; Trandafir et al., 2004). Since vasospasm in the brain often leads to cerebrovascular infarct (stroke), celecoxib may well reduce the risk of stroke via its KCNQ5-mediated influence on the deleterious AVP response. Thus, the ion channel hypothesis put forward by these authors to explain the differential CV risk of celecoxib *vs*. the other COXIB drugs can be summed up like this: acting as a M-channel opener and a VGCC blocker, celecoxib causes vasodilation, a reduction in systemic vascular resistance and a drop in systemic blood pressure, reducing the stress on the heart and vascular system, which lowers the risk of heart attack. In the brain, the reduced tension in the cerebral vasculature, combined with anti-vasospasm activity, reduces the risk of cerebral stroke. Thus, the overall CV risk of COXIBs, which might increase due to differential actions on lipids (*e.g*., the "lipid imbalance theory," see below) is postulated to be balanced by reduction in CV events via direct actions on ion channels. Since rofecoxib is inactive against both KCNQ and VGCC channels, its negative effects are not balanced by the beneficial ones, leading to more adverse CV events.

But this issue begs a brief discussion of why the COXIBs should increase the risk of CV events in the first place. The leading hypothesis is called the "lipid imbalance theory." It is based on the opposing effects on thrombosis of prostinoids consisting of thromboxanes and prostacyclins, thought to be produced by the COX-1 and COX-2 enzymes, respectively (Fitzgerald, 2004). In particular, PGI_2 is a strong vasodilator, and reduces the aggregation response of platelets, whereas thromboxane A_2 is highly thrombogenic. Thus, selective blockade of COX-2 activity would then increase vascular thrombosis in the absence of other ameliorating factors. However pleasing this theory is in its parsimony, though, it may have shortcomings (see Flavahan, 2007 for a nice review). Firstly, the COX subtype responsible for PGI_2 production in the endothelia remains controversial, with data pointing to the COX-1, not the COX-2 enzyme as largely responsible; secondly, platelet activity may well not be increased in patients taking COX-2 inhibitors, and finally, the studies identifying rofecoxib as increasing CV risk did not show that risk decreased by concomitant aspirin use (Bresalier et al.,

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2005), as might be expected if increased thrombogenic activity were responsible. Thus, this fascinating, and important, issue seems to be enigmatic.

Whatever the underlying cause(s) may be of the CV risk of the COXIBs in general, that of celecoxib seems be uniquely lower. Fig. 1 summarizes the hypothesis put forward by Brueggemann et al. to explain this finding. The two ion channel types involved are KCNQ5 K^+ channels, and "L-type" VGCCs. The channel-opening action of celecoxib on the former type retards cellular depolarization in response to excitatory inputs, as do M-type channels in neurons, resulting in VSMCs in less opening of VGCCs and less Ca^{2+} spiking. The inhibitory action is reinforced by direct blockade of the VGCCs, which acts against rises in intracellular Ca^{2+}, Ca^{2+}-induced Ca^{2+} release from intracellular stores, and smooth muscle contraction. Brueggemann et al. suggest that the overall effects are a fall in systemic vascular resistance and blood pressure, and reduced vasospasm in the brain, both of which contributing to fewer heart attacks and strokes, and lower overall CV-mediated morbidity. Is this scenario plausible? Quite definitely. Is it entirely true? Only further study will tell, but this paper indicates that such further investigation is highly warranted. Interestingly, the celecoxib analog 2,5-dimethyl-celecoxib, which has no activity against the COX-2 enzyme (Schönthal, 2006), had the same effect on both channels as did celecoxib, indicating that the ion-channel mechanism reported here as nothing to do with cyclo-oxygenase activity.

This study reinforces the potential therapeutic potential of altering vascular ion channel activity for CV disease. Although Ca^{2+}-channel blocking drugs are currently being used as anti-hypertensives, drugs acting on KCNQ channels have not so far been seriously explored. Probably, this is because the KCNQ2-5 group of channels have been associated with neuronal function, with the vast majority of their study being in the nervous system. However, it is increasingly clear that these channels play critical roles in the vasculature, where their regulation by the myriad signaling pathways worked out in neurons (Delmas and Brown, 2005) likely exerts strong control over vascular smooth muscle tone, and consequently may be important targets for pharmaceutical interventions.

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The celecoxib study presented in this issue may thus be the proverbial "tip of the iceberg," and we will likely be expecting additional discoveries that shed light on the mechanisms conferring safety to anti-inflammatory drugs such as the COXIBs, and on novel modes of therapeutic interventions in general for cardiovascular and cerebrovascular disease.

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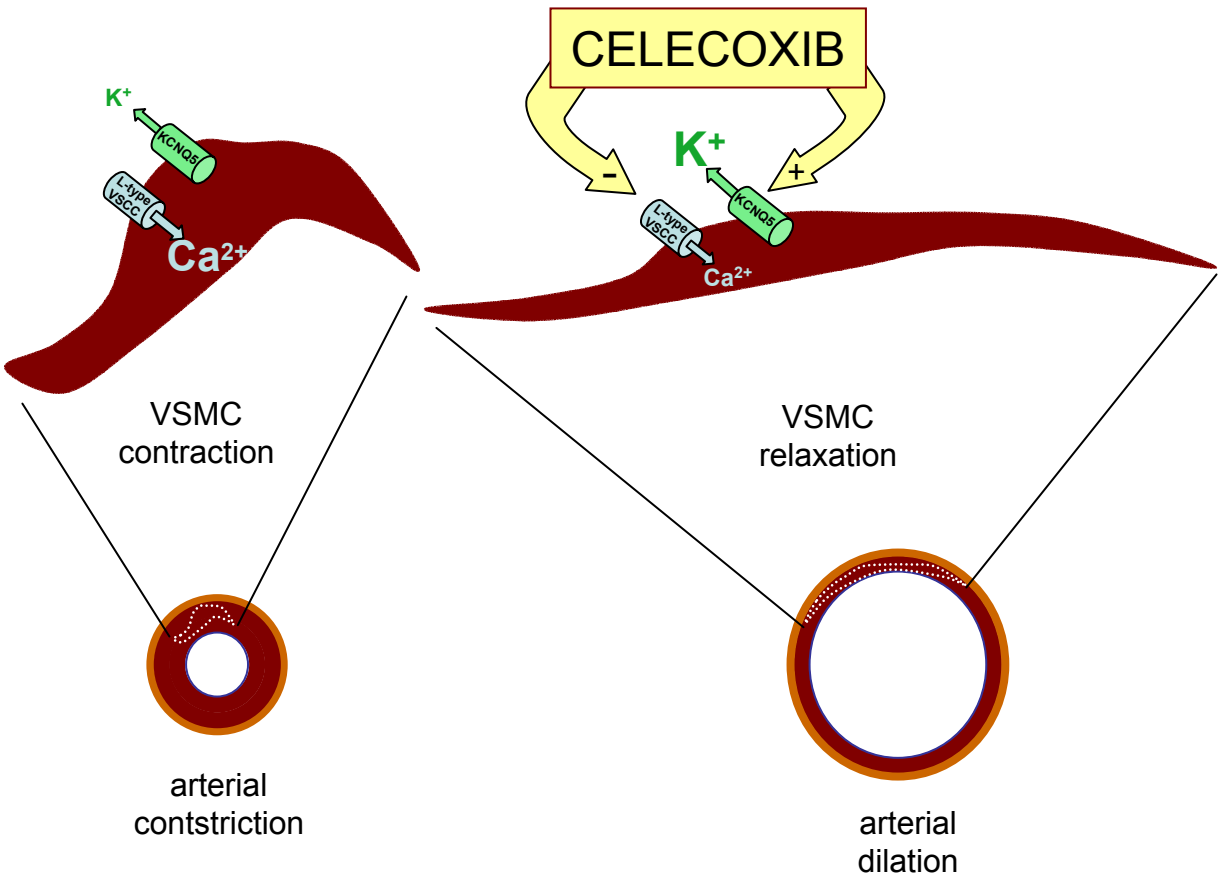
Figure legend.

Fig. 1. Schematic representation of proposed antihypertensive effects of celecoxib. Complementary actions of celecoxib on different classes of ion channels in vascular smooth muscle cells (VSMC) (enhancement of KCNQ5 potassium channel activity and suppression of L-type voltage-sensitive calcium channel activity) result in relaxation of arterial myocytes and vasodilation.

Figure 1


CELECOXIB
K+
KCNQ5
L-type VSCC
Ca2+
-
+
VSMC contraction
VSMC relaxation
arterial contstriction
arterial dilation