Fight or flight: the culprit is lurking in the neighbourhood

Annette C Dolphin and Ivan Kadurin

Department of Neuroscience, Physiology and Pharmacology, University College London, London, UK.

Abstract

The fight-or-flight response is studied by all students of Physiology as a concerted bodily response to danger. Liu et al [1] have now revealed its mechanism, after surveying the proteomic neighbourhood around the cardiac calcium channels in a study which is a tour-de-force of modern biological techniques.

The fight-or-flight response is a classic example of a concerted reaction to exertion or danger, mediated by adrenaline and noradrenaline, involving multiple physiological systems preparing the body to respond. The heart beats faster and its force of contraction is increased. A key mechanism underlying the increase in contractile force involves stimulation of cardiac ß1-adrenergic G-protein coupled receptors (ß-AR), activating adenylate cyclase, stimulating protein kinase A (PKA), and enhancing ventricular myocyte calcium currents [2]. After many years of research by many different groups, Liu et al [1] have now elucidated a surprising mechanism for this response (Fig.1).

Researchers first assumed that this mechanism would involve PKA-mediated phosphorylation of key residues within the pore-forming cardiac calcium channel subunit, α_1C ($Ca_V1.2$), or its predominant auxiliary Cavß2 subunit [3]. A residue in the C-terminus of α_1C, Ser1928, was a promising candidate [3]. Surprisingly, ß1-AR-mediated enhancement of Cav1.2 activity in the heart was not prevented in Ser1928-Ala α_1C knock-in mice [4]. However, stimulation of the ß2-AR-mediated pathway was abolished in hippocampal neurons from these mice [5].

Also, as part of the fight-or-flight response, certain blood vessels dilate due to relaxation of vascular smooth muscle cells (VSMCs), via ß-AR-mediated signalling. However, this cascade generally produces only a modest enhancement of Cav1.2 currents in VSMCs [6]. Nevertheless, Ser1928-Ala α_1C knock-in mice lacked any D-glucose-mediated increase in $Ca_V1.2$ activity in VSMCs, also a PKA-dependent response [7].

Phosphorylation of Ser1700 plus Thr1704 in α_1C [8], combined with proteolytic cleavage of its C-terminus, was also a promising candidate mechanism. Indeed, knock-in mice with mutations of these residues exhibited reduced ß-AR-mediated stimulation, and smaller basal cardiac Ca^{2+} currents [8]. This result was challenged in a subsequent study, which suggested the primary effect of these mutations was on α_1C cell-surface expression [9]. It is possible that effects on channel trafficking and interaction with scaffold proteins may confound interpretation of phosphorylation-site mutations.

Because of these inconclusive results, Liu et al [1] used a transgenic mouse expressing a 1,4-dihydropyridine (DHP) calcium channel antagonist-insensitive α_1C, in which they mutated every consensus PKA phosphorylation site (37 mutations), and another mouse expressing GFP-ß2b with all its PKA sites mutated (28 mutations). Crossing these mice, they found the DHP-insensitive (i.e. transgenic) calcium currents in their cardiomyocytes still responded to ß-AR stimulation.

They then used the novel technique of proximity proteomics to identify proteins in the vicinity of the α_1C channel complex, surmising that an inhibitory protein might reduce its interaction after phosphorylation, or alternatively a stimulatory protein might associate more readily. This technique

required transgenic mice with ascorbate peroxidase (APEX2) fused to either α_1C or ß2b, allowing biotin to be conjugated to nearby proteins, allowing subsequent identification by mass spectrometry.

Using this method, they found that the small G-protein Rad stood out as exhibiting reduced association with the channel complex in isoprenaline-activated cardiomyocytes from α_1C-APEX mice (by 50%) as well as in isoprenaline-perfused whole hearts (by 36%), and in cardiomyocytes from ß2b-APEX mice (by 30%). Rad was one of the few proteins whose biotinylation was consistently reduced in all three APEX mice experiments. The technique was also validated by an increase in biotinylation of the PKA catalytic subunit following ß-AR stimulation, suggesting it was recruited to the complex.

Not surprisingly the authors homed in on Rad, because it was already known to affect calcium channel function. Rad is a member of the RGK family of small G-proteins, also including Rem and Gem/Kir, which have homology to Ras. Previous studies have shown RGK proteins to profoundly inhibit calcium channel function by binding to Ca_Vß subunits and interfering with the ability of Ca_Vß to interact with the α_1 subunit. The physiological relevance of this effect was initially unclear [10], although overexpression of Rem prevented cyclic AMP-mediated stimulation in cardiomyocytes [10].

Liu et al. [1] then succeeded in reconstituting the elusive cyclic AMP-mediated stimulation of α_1C/ß2b currents in HEK293T cells, by including a small ratio of Rad, such that the tonic inhibition it produced could be reversed by forskolin, which directly activates adenylate cyclase. They then showed this reversal required Rad phosphorylation, which prevented its interaction with ß2b. A number of key residues on Rad are phosphorylated by PKA, and their mutation prevented forskolin-mediated stimulation. Rad also has a polybasic C-terminal region, mediating its association with the plasma membrane, via interaction with phospholipids such as PIP_2, which was also disrupted by PKA phosphorylation [1] (Fig.1).

Liu et al [1] also provide preliminary evidence of a similar mechanism for $Ca_V1.3$ and $Ca_V2.2$, and that Rem can also mediate this effect [1]. Indeed, RGK proteins are known to bind to other ß subunits, and thus this pathway appears potentially to be universal for all Ca_V1 and Ca_V2 channels. Surprisingly, PKA-mediated enhancement of Ca_V2 channels is not a prominently observed effect in neurons, possibly indicating lack of co-localization with RGK proteins. Furthermore, the detailed mechanism of how PKA modulates Cav1.2 channels in smooth muscle also remains to be established. Future studies will certainly reveal the underlying causes for differential, tissue-specific regulation of this pathway in heart, brain and VSMCs, and the roles of scaffolding proteins such as A-kinase-anchoring proteins.

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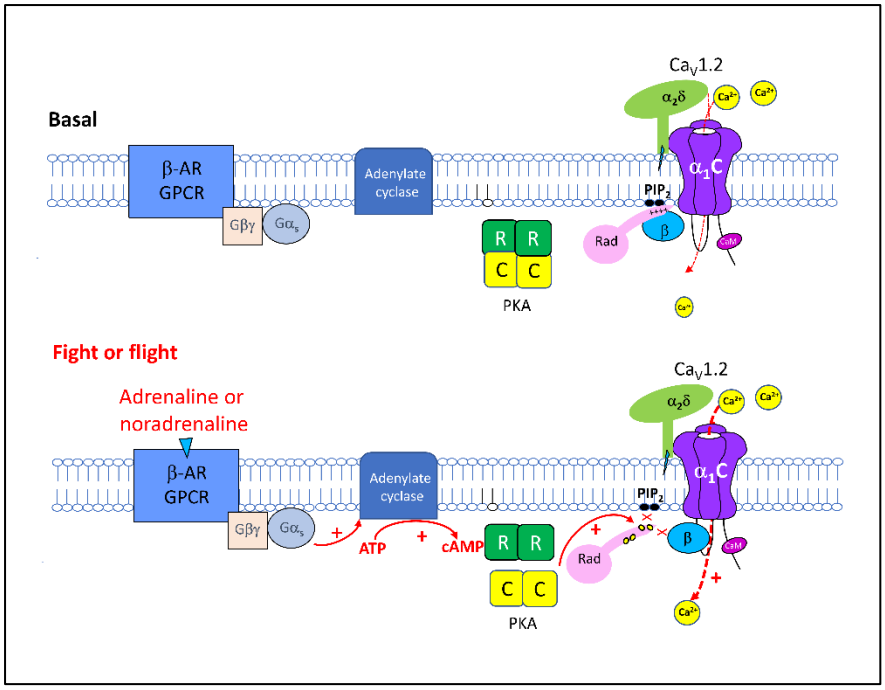

Figure 1: Schematic of pathway vealed to be involved in the fight-or-flight response.

The upper panel is the baseline condition, and the lower panel shows the effect on the pathway of stimulation of the ß-AR [1].