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COMMENTARY

Does levosimendan act as a Ca^{2+} sensitizer or PDE3 inhibitor?: Commentary on Orstavik *et al.*, Br J Pharmacol 171: 5169–5181

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LINKED ARTICLE

This article is a Commentary on Orstavik O, Ata SH, Riise J, Dahl CP, Andersen GO, Levy FO, Skomedal T, Osnes J-B, and Qvigstad E (2014). PDE3-inhibition by levosimendan is sufficient to account for its inotropic effect in failing human heart. Br J Pharmacol 171: 5169–5181. doi: 10.1111/bph.12647

Abbreviations

CRC, concentration-response curve; PIE, positive inotropic effect

This is a Commentary on an article in BJP by Orstavik *et al.*, 2014; 171: 5169–5181. Calcium ions (Ca^{2+}) play a key role in cardiac excitation–contraction coupling. Therefore, cardiac contractility is crucially regulated by modulation of cardiac Ca^{2+} signalling, which is classified into three processes. A transient increase in intracellular Ca^{2+} concentration subsequent to membrane excitation (upstream mechanism) can be detected as the Ca^{2+} transient by application of Ca^{2+} sensitive bioluminescent protein aequorin or fluorescent dye such as indo-1or fura-2. Ca^{2+} binding to troponin C plays a central role by triggering the activation of thin (actin, tropomyosin, troponin) and thick (myosin) filament (central mechanism). The process subsequent to Ca^{2+} binding to troponin C leading to thin and thick filament interaction (downstream mechanism) is not easy to experimentally differentiate from the central mechanism in intact myocardial cells.

There are three main mechanisms whereby cardiac contractility can be increased. β-Adrenoceptor stimulation and/or inhibition of PDE that hydrolyses cAMP to 5'-AMP, both increase the intracellular accumulation of cAMP, which then activates PKA and increases cardiac contractility; this constitutes an upstream mechanism. In contrast, Ca^{2+} sensitizers act via a central and/or downstream mechanism (Blinks and Endoh, 1986). Cardiotonic agents that act through the latter mechanism have the added advantage of being devoid of unwanted effects induced by intracellular Ca^{2+} overload; this readily occurs with agents acting via the upstream mechanism and can lead to severe cardiac arrhythmias and ultimately myocardial cell death. In addition, Ca^{2+} sensitizers have another advantage in that they do not need activation energy, which is required for Ca^{2+} transport through myocardial cell membrane.

During the course of an extensive effort to develop drugs for the treatment of heart failure to replace catecholamines and the classical cardiac glycoside digitalis, levosimendan was developed as a novel cardiotonic agent that acts as a Ca^{2+} sensitizer (Endoh, 2002). Levosimendan induces its Ca^{2+} sensitizing action by stabilizing the Ca^{2+}-troponin complex (Haikala *et al.*, 1995), but it is also a potent PDE3 inhibitor (Endoh, 2002). Therefore, it is contentious whether levosimendan produces its beneficial effects, in experimental

animal models and patients with heart failure, by acting as a Ca^{2+} sensitizer, PDE3 inhibitor or a combination of both.

In the paper in question, Orstavik and co-workers have concluded that the inhibition of PDE3 is sufficient to account for its positive inotropic effect (PIE) on failing human hearts (Orstavik *et al.*, 2014). Experiments were performed using ventricular strips isolated from failing human hearts and normal male Wistar rats. The most important finding is that the PIE of levosimendan is abolished by the PDE3 inhibitor cilostamide, but enhanced by the combination of isoprenaline and the PDE4 inhibitor rolipram. In most previous studies, based on circumstantial evidence, it has been concluded that levosimendan's Ca^{2+} sensitizing action is the primary mechanism whereby it induces its PIE. Hence, the current paper by Orstavik *et al.* (2014) is unique and important in that it demonstrates the significant and predominant role of PDE3 inhibition in the PIE of levosimendan in human failing ventricular myocardium, with Ca^{2+} sensitization only having, if any, a minor role. In addition, the PIE of levosimendan was found to be associated with a positive lusitropic effect over the whole concentration range examined; this may be elicited via a cAMP-mediated mechanism.

It has been shown that muscarinic receptor stimulation can be used as an excellent pharmacological tool to differentiate the PIE mediated by cAMP-dependent signalling from cAMP-independent mechanisms in mammalian ventricular myocardium (Endoh *et al.*, 1986; Endoh, 2002). In this context, it is noteworthy that, consistent with the findings of Orstavik and co-workers, (2014), the muscarinic receptor agonist carbachol is able to abolish the PIE and the increase in Ca^{2+} transients induced by levosimendan, implying that cAMP-mediated signalling stimulated by the accumulation of cAMP resulting from PDE3 inhibition plays a crucial role in the PIE of levosimendan in rabbit and canine ventricular myocardium (Sato *et al.*, 1998; Takahashi and Endoh, 2005). Previous findings have also suggested that the Ca^{2+} sensitizing effect of levosimendan is mediated by an enhanced myofilament response to the increased Ca^{2+} transients resulting from PDE3 inhibition (Endoh *et al.*, 1986).

In the current study it was shown that, in contrast to ((+)-5-[1-(3,4-dimethoxybenzoyl)-1,2,3,4-tetrahydro-6-quinolyl]-6-methyl-3,6-dihydro-2H-1,3,4-thiadiazin-2-one) (EMD57033) that shifted the concentration–response curve (CRC) for Ca^{2+} to the left, levosimendan does not affect the CRC for Ca^{2+} in rat ventricular strips. From this the authors concluded that levosimendan does not have a Ca^{2+} sensitizing action at the level of the myofilaments. However, it has to be kept in mind that the contribution of a Ca^{2+} sensitizing mechanism to PIE is debatable (Endoh, 1998; 2002; 2008). While it has been repeatedly shown that levosimendan acts by the central mechanism (Haikala *et al.*, 1995), the effect of EMD57033 is primarily exerted at the level of myosin, that is via the downstream mechanism (Solaro *et al.*, 1993; Endoh, 1998). Although the observations in rat ventricular strips appear to corroborate those obtained in human ventricular tissues, it is difficult to definitively conclude that Ca^{2+} sensitization does not contribute to the PIE of levosimendan, as Ca^{2+} transients were not detected in the current study. It is generally thought that a Ca^{2+} sensitizing effect can only be detected by examining the relationship between contractile force and Ca^{2+} transients.

Furthermore, in order to exclude the potential contribution of an upstream mechanism, the myofilament Ca^{2+} sensitivity could be examined in skinned fibres rather than intact myocardium. As intact ventricular strips isolated from rats were used in the present study, it is possible that effects induced by an upstream mechanism affected the exact determination of the Ca^{2+} sensitizing effect of levosimendan.

Levosimendan at the highest concentration (10^{-5} M) examined in this study elicited a cardiodepressive effect, indicating that the CRC for levosimendan is bell-shaped (Figure 1 of Orstavik *et al.*, 2014). In this context, it is noteworthy that in previous studies, it was shown that the bell-shaped CRC for inotropic effects is due to a decrease in myofilament Ca^{2+} sensitivity induced by levosimendan at high concentrations (Takahashi and Endoh, 2005). Therefore, it would seem the inotropic effect of levosimendan is mediated by three mechanisms, namely: (i) PDE3 inhibition; (ii) Ca^{2+} sensitization; and (iii) Ca^{2+} desensitization in intact mammalian myocardium.

Another clinically available cardiotonic agent, pimobendan, that has similar Ca^{2+} sensitizing and PDE3 inhibitory actions, has been shown to increase survival in an animal model of heart failure, in which the genetic modulation of troponin induced cardiomyopathy by decreasing Ca^{2+} sensitivity (Du *et al.*, 2007). In this heart failure model, it was clearly shown that the pure PDE3 inhibitor amrinone did not benefit survival and even shortened survival time at higher concentrations, that elicited a harmful effect. As the final goal of the development of novel cardiotonic agents is to prolong the life of patients with chronic congestive heart failure, it is important and of great interest to examine the effectiveness of levosimendan in comparison with other PDE3 inhibitors, such as amrinone or cilostamide, on survival in this genetically-evoked animal model of heart failure.

An important aspect that has to be kept in mind in the clinical application of levosimendan is its unique pharmacokinetic properties, as emphasized by the authors (Orstavik *et al.*, 2014). Levosimendan is metabolized finally to an effective active metabolite OR-1896 with a notably long half-life *in vivo*. The mechanism of the PIE induced by this metabolite is very similar to that of levosimendan, that is Ca^{2+} sensitization and PDE3 inhibition (Takahashi and Endoh, 2002). Also, it is highly likely that this active metabolite contributes significantly to the PIE of levosimendan *in vivo*.

Extensive efforts to develop cardiotonic agents with novel mechanisms of action started in the late 1970s. However, as yet few new compounds are available for effectively treating patients with chronic congestive heart failure. While it was clearly shown that PDE3 inhibition plays a crucial role in the PIE of levosimendan in isolated ventricular myocardium in the current study, the clinical effectiveness of cardiotonic agents mechanistically possessing Ca^{2+} sensitizing action, such as pimobendan, levosimendan, and the myosin activator omecamtiv mecarbil (Cleland *et al.*, 2011), awaits further research. PDE3 inhibitors are used for the treatment of contractile dysfunction in patients with acute congestive heart failure, but there is still a need to develop novel effective agents that act through the central and/or downstream mechanism, for establishing life-saving long-term pharmacotherapy of chronic heart failure patients in the future.

Conflict of interest

None.

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