

PHARMED INSTITUTE OF CYBERNETICS

PIC Res. Comm. 1/2011
(rev. 9/2010)

A New Model on the Mechanism of Action of Aspirin: Ca²⁺ Efflux Inhibitor

Rainer K. Liedtke, MD
PIC, Munich, Germany, liedtke@pharmed.de

Key Words. Aspirin, NSAID, Intrinsic and extrinsic action, oxydative phosphorylation, cytosolic calcium ions, calcium pumps, calcium efflux inhibitor

Abstract. A new model of Aspirin's mechanism of action is presented. It indicates that the intrinsic property results from energetic consequences of an inhibition of the mitochondrial oxydative phosphorylation. This initial action is transformed downstream into an imbalance of the interplay of the ion-channels which influence the calcium ion transport. The result is an increase of the cytosolic free calcium ions. Based on this model Aspirin acts functionally as a cellular calcium efflux inhibitor. Aspirin's inhibition of the cyclooxygenase enzymes appears an extrinsic property which provides accentuating effects. It is assumed that this dualistic action represents a mechanism of action of some other non-steroidal anti-inflammatory drugs.

Introduction

The effect of Aspirin on the synthesis of prostaglandins (PG) via a COX inhibition (1) has reached a central position for explanations of its basic mechanism. However, for Aspirin (ASA) exists a broad diversity of different biochemical findings and pharmacological effects: Uncoupling of the mitochondrial oxidative phosphorylation, inhibition of various enzymes such as e.g. cyclooxygenases (COX), dehydrogenases, transferases, decarboxylases, effects on glucose, blood vessels, blood clotting, liver, kidney, stomach, inner ear. Although a considerable amount of work exists on the COX inhibition, in particular on aspects of inflammation, the view that only this specific enzyme reaction is responsible for Aspirin's diversity of effects is still controversial. Amongst several other clinical actions, such effects as e.g. its induction of a tinnitus, the plausibility as its sole cause of analgesic effects is debated.

A major aspect in this problem appears to be to delineate intrinsic and extrinsic (relational) effects. Aspirin, and the most of the non-steroidal anti-inflammatory drugs (NSAID) uncouple the energetic core process of the mitochondrial oxidative phosphorylation (OXPHOS). Therefore we have already evaluated, in a theoretic-exemplary manner, some respective consequences which can occur due to this basic effect. In a first model we examined potential causes of vascular NSAID effects [2], and in a second model whether and how an inhibition of the OXPHOS can be correlated with an analgesic effect [3]. The conclusions derived from these models were: The OXPHOS inhibition of NSAIDs can (a) initialize an increase in the intracellular formation of calcium phosphate complexes. These can induce a consecutive cascade of chemical processes which effect a vascular biomineralization and respective clinical consequences. (b) The OXPHOS inhibition of NSAIDs can induce shifts in the intracellular ionic composition which on their part effect a change of the relative ionic composition in the extracellular fluid. This ionic shift influences, according to the Hodgkin-Huxley model [4], the membrane potential of adjacent nerve fibers, thus affects their excitability. A peripheral analgesic effect can be explained through a changed excitability pattern of the neuronal transmission, as discussed in detail elsewhere [3].

Based on these preceding findings it was the aim to specify in a further model the consequences of an NSAID induced OXPHOS inhibition on the cytosolic calcium ions (Ca²⁺ (i)). It shall describe, in a qualitative manner, the resulting functional interplay of the OXPHOS inhibition in the cytosol. The model has been prepared by means of a modified entity relationship modeling (BMA/3), and with reference to experimental data from the literature.

Arrangement of the Basic Processes of the Model

Premises: In sum, the uncoupling/inhibition of the OXPHOS leads to a reduced synthesis of Adenosine-5-Triphosphate (ATP) of the ATP-Synthase (complex V), thus effects a lower intracellular ATP concentration (ATP(i)). Functionally, a low ATP(i) means a reduced activity of all ion pumps that receive their main energy from the ATP hydrolysis. The Ca²⁺(i) are removed by ATP dependent pumps, and indirectly as to ATP-energy, by the electrogenic sodium-calcium exchanger (NCX). The ATP dependent pumps include primarily two Ca-ATPases: The plasma membrane Ca²⁺-transporting ATPase (PMCA), and the sarco/endoplasmic reticulum Ca²⁺ ATPase (SERCA). The latter controls the Ca²⁺(i) balance via compartmentation (endoplasmic reticulum pools). Moreover, functionally involved is also the ATP-driven sodium/potassium exchanger Na⁺/K⁺-ATPase (NaKA). In the whole the process of removing calcium can be described as a 'calcium cycle', a dynamic interplay for the maintenance of a low Ca²⁺(i) which includes as operational components ATP(i), the Ca-ATPases (PMCA, SERCA), the NCX and the NaKA.

Functional Aspects. The PMCA is powered by the hydrolysis of ATP with a stoichiometry of one Ca²⁺ removed for each molecule of ATP hydrolysed. This pump has a higher Ca²⁺ affinity but lower capacity than the NCX. Therefore the PMCA is functionally suited for maintaining the normally low intracellular calcium concentration. The SERCA acts in a complementary manner as interim storage to deposit and/or mobilize Ca²⁺(i) if needed. The electrogenic antiporter NCX is using the energy of the Na⁺ gradient and links movements of Ca²⁺ to the reciprocal movement of Na⁺. Although primarily regulated by transported Na⁺ and Ca²⁺, the NCX is also influenced by protons and the metabolic state of the cell [5]. There exists some evidence for a coupling between inward movement of Ca²⁺ and outward movement of Na⁺ [6]. The NCX has a lower Ca²⁺ affinity than the PMCA, but transports rapidly with a higher capacity, thus is suited for higher Ca²⁺ concentration. When the levels of intracellular Na⁺ (Na⁺(i)) critically rise the NCX begins importing Ca²⁺. That implies a reduction of outward movement of Ca²⁺, when the NaKA activity is decreased, i.e. at high intracellular Na⁺. Depending on the Na⁺ and Ca²⁺ gradients the NCX may operate forward and in reverse direction simultaneously. Since this transport is electrogenic by altering the membrane potential, a depolarization of the membrane can reverse the direction. Thus, in sum the activities of the PMCA and NCX pumps complement each other.

Mechanism (Figure 1): Formally an accumulation and overload of Ca²⁺(i) can result from an imbalance of the parameters Ca²⁺ influx, endoplasmic storage of Ca²⁺, and Ca²⁺ efflux. Since Aspirin, and other NSAID, induce via OXPHOS inhibition a low ATP(i), the shortage of utilizable ATP energy inhibits the functions of the ATP-dependent pumps. The low ATP(i)

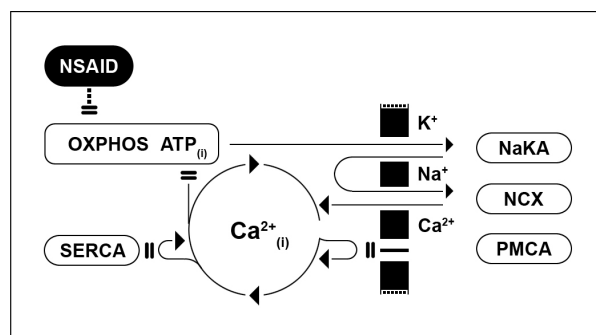


Figure 1: Model on consequences of an NSAID induced inhibition of the mitochondrial oxydative phosphorylation. The resulting imbalance in the interplay of the ion channels involved in the Ca²⁺ transport induces an inhibition of cytosolic calcium efflux. Details see text. Abbreviations: SERCA: (Sarco)Endoplasmic Ca²⁺-ATPase, PMCA: Plasma Membrane Ca²⁺-ATPase; NAKA: Na⁺/K⁺ ATPase, NCX: Na⁺/Ca²⁺ Exchanger, OXPHOS: Oxydative Phosphorylation, ATP(i): intracellullar ATP, Ca²⁺(i): Cytosolic calcium ions; ■ : Plasma Membrane; = : Inhibition)

effects a NaKA channel opening, thus an increased K⁺ outflow and increased Na⁺ inflow. The increase in intracellular sodium ions effects on its part an increase of Ca²⁺(i). With the decreased NaKA activity occurs also a reduced outward movement of Ca²⁺. That is based on a coupling of the NCX between inward movement of Ca²⁺ and outward movement of Na⁺, since the NCX begins importing Ca²⁺ when Na⁺(i) rise beyond a critical point. Due to the low ATP(i) the capacity of the ATP-dependent Ca²⁺-pumps is reduced. The remaining activity of the PMCA to remove Ca²⁺(i) is insufficient to counterbalance the intracellular Ca²⁺ accumulation, and the lowered capacity of the SERCA can not compensate this by Ca²⁺(i) compartmentation in the endoplasmic reticulum pools. Consequently free calcium ions accumulate in the cytosol. The resulting Ca²⁺(i) overload effects on its part a vicious circle, since it creates a further decrease in mitochondrial ATP synthesis.

Therefore the model indicates an imbalance in the Ca²⁺ inward/outward flow: Due to the NSAID induced ATP energy shortage the Ca²⁺ influx is in sum higher than its compensation trough cellular Ca²⁺ compartmentation and efflux. Thus the NSAID acts functionally as inhibitor of the cellular Ca²⁺ efflux.

Discussion

Aspirin actions can contribute on different metabolic levels to an OXPHOS inhibition, directly or indirectly: Mitochondrial effects which are in context with a calcium overload have been found in both liver and kidney mitochondria [7][8]. The addition of Ca²⁺ to Aspirin led to a potentiation of the impaired mitochondrial ATP synthesis [9]. A Ca²⁺(i) accumulation may also be effected by a direct reaction of Aspirin with the PMCA [10]. In addition, Aspirin can inhibit the NaKA and Mg²⁺-ATPase [11]. Also a perturbation of the intracellular calcium by inhibiting the endoplasmic reticulum Ca²⁺-ATPases by the NSAID Celecoxib has been described [12]. Moreover, like most other NSAID, Aspirin can react with enzymes of the citrate cycle, such as e.g. the alpha-ketoglutarate dehydrogenase [13].

In sum all such interventions induce or intensify a mitochondrial permeability transition, a shift in the intracellular ionic composition, and a pro-apoptotic release of Cytochrome C, as already discussed in detail elsewhere [2]. Therefore, the mechanism of Aspirin appears to be initialized via its OXPHOS inhibition. This action is then transformed downstream into the effect of a cytosolic calcium efflux inhibition. The expression of an analgesic effect is inherent in this process, and can be explained by the induced shift of the relative ionic composition as discussed in detail elsewhere [3].

This intrinsic mechanism of action however does not prevent or exclude the occurrence of other pharmacodynamic effects. Therefore the mechanism of action appears to be dualistic one. In order to delineate these effects, extrinsic (relational) actions may be designated as "shell effects". They contribute to an accentuated pharmacodynamic profile and are primarily determined by specific components of the chemical structure of the NSAID, and/or their metabolites. Therefore these represent more specific reactions, such as e.g. the inhibition of COX enzymes. Aspirin's inhibition of the thromboxane synthesis in platelets may indicate an example of such extrinsic effect.

It is also assumed that the intrinsic property within the NSAID group will be essentially the same, whereas their extrinsic effects may differ, e.g. due to their chemically different affinity to the COX isomers. This can also explain that some NSAID enantiomers which effect a different extent of PG inhibition can show the same extent of analgesic effects.

Conclusions

The new model leads to the conclusion that in sum the Aspirin induced Ca²⁺ influx is higher than its compensation trough cellular Ca²⁺ compartmentation and efflux. Pharmacologically the mechanism of action of Aspirin can be formally subsumed as a cellular calcium efflux inhibitor as intrinsic property. The COX inhibition of aspirin appears to represent an extrinsic (relational) property which induces, on a secondary level, a number of actions which provide an accentuation of the effects. This kind of dualistic action may also be common mechanism of the group of NSAID.

References

1. Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature New Biol* 1971, 23: 232-235
2. Liedtke RK. A model on the induction of adverse vascular long-term effects of NSAIDs. *Med Chem* 2009, 5(1): 23-28
3. Liedtke RK. A general theory on pain as an integrated thermodynamic mechanism. *Med Hypotheses* 2009, 73: 86–89
4. Hodgkin AL, Huxley AF. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J Physiol* 1952, 117: 500-544
5. DiPolo R, Beaugé L. Sodium/calcium exchanger: influence of metabolic regulation on ion carrier interactions, *Physiol Rev* 2006, 86 (1): 155-203
6. Baker PF, Blaustein MP, Hodgkin AL, Steinhardt RA. The influence of calcium on sodium efflux in squid axons. *J Physiol* 1969, 200(2): 431-58
7. Petrescu I, Tarba C. Uncoupling effects of diclofenac and aspirin in the perfused liver and isolated hepatic mitochondria of rat. *Biochim Biophys Acta* 1997, 1318: 385-94
8. Mingatto FE, Santos AC, Uyemura SA, Jordani MC, Curti C. In vitro interaction of nonsteroidal anti-inflammatory drugs on oxidative phosphorylation of rat kidney mitochondria: respiration and ATP synthesis. *Arch Biochem Biophys*, 1996, 334: 303-8
9. Tomoda T, Takeda W, Kurashige T, Enzan H, Miyhara M. Acetylsalicylate-induced mitochondrial dysfunction and its potentiation by Ca²⁺. *Liver* 1994, 14: 103-8
10. Omer B, Oner P, Baysal K, Oz H. Effect of acetylsalicylic acid on liver plasma membrane Ca²⁺ ATPase activity. *Pol J Pharmacol Pharm* 1990, 42: 441-6
11. Sarkar AK, Charaboti A, Saha UK, Bose SK, Sengupta D. Effects of aspirin and paracetamol on ATPases of human fetal brain: an in vitro study. *Indian J Exp Biol* 1989, 27(9): 802-4
12. Johnson AJ, Hsu AL, Lin HP, Song X, Chen CS. The cyclo-oxygenase-2 inhibitor celecoxib perturbs intracellular calcium by inhibiting endoplasmic reticulum Ca²⁺-ATPases: a plausible link with its anti-tumour effect and cardiovascular risks. *Biochem J* 2002, 366: 831-7
13. Persson AC, Szweda LI, Sadek HA. Inhibition of cardiac mitochondrial respiration by salicylic acid and acetylsalicylate. *J Cardiovasc Pharmacol* 2004; 44(5): 591-5