Aspirin-like drugs inhibit neuronally evoked responses in isolated guinea-pig ileum

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Summary. - The acute effects of non steroidal anti-inflammatory drugs (NSAIDs) were studied on guinea-pig ileum isolated preparation. Two cyclo-oxygenase inhibitors, aspirin and indomethacin, and two NSAID devoid of this effect, salicylic acid and benzydamine, were injected into the bath 1 min before PGE1 or CCK-8. All drugs tested elicited a dose-related inhibition of the neuronally evoked contractile responses to submaximal dose of PGE1 and CCK-8. These drugs depressed also the opioid system(s) activated by PGE1 or CCK-8. These results indicate that the inhibition of neuronally evoked response is a common mechanism of NSAIDs. This mechanism may have an important role on analgesic and anti-inflammatory action of these drugs.

Keywords: guinea-pig ileum, aspirin, indomethacin, prostaglandin.

Riassunto. - I farmaci aspirino-simili inibiscono le risposte neuronali nell’ileo di cavia. - Gli effetti acuti degli antinfiammatori non steroidal (NSAIDs) sono stati studiati su preparati isolati di cavia. Due inibitori della cicloossigenasi, aspirina e indometacina, e due NSAID senza questa capacità, acido salicilico e benzidamina, sono stati posti nel bagno 1 min prima della PGE1 o della CCK-8. Tutti i farmaci testati determinano una inibizione correlata alla dose delle risposte contrattili neuronal per dosi submaximali di PGE1 e CCK-8. I farmaci inibivano anche l’attivazione del sistema oppioide attivato dalla PGE1 o dalla CCK-8. I risultati indicano che l’inibizione di risposte neuronal è un meccanismo comune agli NSAIDs. Tale meccanismo può avere un ruolo importante nell’azione analgesica e antinfiammatoria di questi farmaci.

Parole chiave: ileo di cavia, aspirina, indometacina, prostaglandine.

Introduction

Aspirin-like drugs are believed to exert their anti-inflammatory and analgesic activities by inhibition of cyclo-oxygenase system, particularly in damaged tissues [1-3]. However, there is not a satisfactory correlation between prostaglandin (PG) synthesis inhibition and analgesic effects [4, 5]. Some non-steroidal anti-inflammatory drugs (NSAIDs), salicylic acid included, do not inhibit prostaglandin production at analgesic concentration [6-8]. In addition, many endogenous substance besides PGs are involved in the inflammatory processes [9, 10] and there is not direct evidence for an involvement for PGs in the pharmacological action of these substances. PGs seem to play a modulatory role on only few endogenous substances involved in inflammation and pain [11-13].

It would be therefore very useful to find out whether NSAIDs share some common properties, which could be involved in their analgesic an anti-inflammatory actions. In the present study we investigated the effects of aspirin (ASA) and indomethacin (IND), two cyclo-oxygenase inhibitors and of salicylic acid (SA) and benzydamine (BZ), which are devoid of this action [14, 15], on isolated guinea-pig ileum contracting activity of PGE1 and cholecystokinin (CCK-8). The aim was to verify if NSAIDs could interfere also with the neuronal activity of PGs themselves and other endogenous substances involved in the inflammatory and analgesic processes. Inflammation mediators may facilitate the release of neurotransmitters and these neuronal mechanisms may contribute to the inflammatory and analgesic reactions. In order to avoid any involvement of PGs synthesis inhibition in the observed effect, the drugs were injected into the bath 1 min before the excitatory substances.

The effect of NSAIDs was also examined on neuronal activation of opioid mechanisms by CCK-8 and PGE1 [16, 17].

Methods

Male guinea-pigs weighing 300-400 g were stunned by a blow on the head and exsanguinated. The ileum was removed, discarding the 10 cm nearest the cecum and cleaned. Pieces of ileum 2-3 cm long, from the same animal, were set up under 1 g tension in 10 ml organ baths containing tyrode solution. The bath were maintained at 37 °C and bubbled with a gas mixture composed of 95% O2. The segments were allowed to equilibrate for 30-40 min without washing and their response to ACh (10^-6M) was determined two or three times to ascertain their
suitability and to express the magnitude of the contraction as a percentage of ACh maximum.

Tension changes were recorded by an isotonic force transducer connected with a pen recorder (Ugo Basile, model 7050, Italy). After a further 20-30 min resting period the tissues were exposed to the excitatory peptide CCK-8 or to PGE1.

The effects of IND (2.7 x 10^{-6}M), ASA (5.5 - 11 x 10^{-5}M), SA (6.2 - 12.4 x 10^{-5}) and BZ (3 - 6 x 10^{-7}M) were examined on the submaximal responses elicited by CCK-8 (10^{-9}M) and PGE1 (5 x 10^{-9}M), on their maximal responses obtained at 10^{-8}M and 1.5 x 10^{-8}M. The drugs were injected into the bath 1 min before the excitatory substances.

To assess if PGE1 could activate the opioid system, as CCK-8 and other indirectly acting peptides, naloxone (N, 5.5 x 10^{-7}M) was placed in the bath in the declining phase of the contractile response elicited by PGE1.

Each experiment was performed in at least five tissue from different animals.

Results

Guinea-pig ilea exposed to a concentration 10^{-9}M of CCK-8 responded with a contracture about 50% smaller than the maximal one. The isolated preparations gave reproducible responses following exposure to CCK-8, after washing and a resting period of about 30 min (Fig. 1). Therefore the same tissue was used for many tests so that the different drugs or drug concentrations could be examined under the same experimental conditions. ASA decreased in dose-related way the contraction elicited by CCK-8 and at the higher dose (11 x 10^{-5}M) completely blocked the response.

As shown in Fig. 2, IND (2.7 x 10^{-6}M) inhibited the peptide contraction of about 75%. After washing and a resting period of 30 min the tissue did not recover and the isolated preparation was discarded.

SA reduced in dose-related manner (6.2 - 12.4 x 10^{-9}M) the amplitude of the contractile response to a low dose to CCK-8. The tissue recovered completely after the exposure to this drug (Fig. 3).

BZ inhibited the CCK-8 contractile response on the isolated guinea-pig ileum at very low concentration (3 - 6 x 10^{-7}M) (not shown).

As shown in Fig. 4, the administration of PGE1 at the concentration of 2.8 x 10^{-8}M elicited a contraction of the ileum consisting of two components: the phasic component lasting a few second and the tonic response, which declined more slowly. At this concentration the response was maximal and 70-80% of ACh maximum. The addition of naloxone (5 x 10^{-7}M) in the declining phase caused an excitatory response. A concentration of

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![Fig. 1. - Dose-related reduction by aspirin (ASA, b and d, 5.5 x 10^{-5}M and 11 x 10^{-5}M, respectively) of the excitatory response to cholecystokinin (CCK-8, 10^{-9}M). ACh max: acetylcholine 10^{-6}M; ▲ washout.](image-url)

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Fig. 2. - Reduction by indomethacin (IND, 2.7 x 10^{-6} M) of the excitatory response to cholecystokinin (CCK-8, 10^{-6} M).
ACh max: acetylcholine 10^{-6} M; □: washout.

PGE1 five-fold lower elicited a contraction about 50% smaller than the maximal one. The subsequent of naloxone, at variance to what happened with the low dose of CCK-8, almost always caused a response proportional to the one elicited by PGE1.

ASA (5.5 - 11 x 10^{-5} M) elicited a dose-related reduction of both contractile response (Fig. 4). IND (2.7 x 10^{-6} M) completely blocked the responses to PGE1 and to naloxone (Fig. 5). In some tissue the drug inhibited also the spontaneous activity and the isolated preparations didn’t recover.

SA reduced or blocked the contractile responses (Fig. 6), as did BZ, at the doses previously used (not shown).

BZ was able to reduce in dose-related way also the maximal contractions obtained with CCK-8 or with PGE1 and the one obtained by the subsequent injection of naloxone. ASA, IND and SA, on the other hand, didn’t affect the maximal contractile response to the excitatory substances, but inhibited the contraction to naloxone (not shown).

Discussion

The results of the present study indicate that aspirin, indomethacin and salicylic acid, at doses equal or lower than those effective to block PGs synthesis, are able to inhibit the submaximal contraction responses elicited by CCK-8, confirming previous results [13], and those caused by PGE itself. This inhibitory activity on neuronally evoked response of endogenous substances may play an important role in the analgesic and anti-inflammatory effects of NSAIDs.

With regard to NSAIDs, a distinction must be made between antinociceptive, anti-inflammatory and analgesic effects, the latest being secondary to an inflammatory response. The assumption that these drugs relieve inflammation and pain through an inhibition of PGs synthesis is not a satisfactory explanation. The PGs synthesis inhibition seems to play a dominant role in the anti-inflammatory action and in the gastrointestinal toxicity of aspirin-like drugs (3, 5). However, there is not a close correlation between the capacity of NSAIDs of inhibiting the synthesis of PGs and their capacity of reducing inflammation. In addition, recent observations indicate that peripheral inflammation can be centrally inhibited by the alfa-melanocyte-stimulating hormone neuropeptide [18] and by some NSAIDs such as salicylate and that PGs are not important to central modulation of inflammation [19]. With respect to the analgesic activity, several papers have appeared which brought forth arguments against a purely peripheral mode of action of NSAIDs. Although in vivo studies have indicated that some of these drugs when systemically administered can inhibit rat brain PGs synthesis [19, 20] and that nociceptive information may be facilitated by PGs sensitive mechanisms in the SNC, the pain related role of PGs remain unclear [4].
Fig. 3. - Dose-related reduction by salicylic acid (SA, b and d, $6.2 \times 10^{-5}$M and $12.4 \times 10^{-5}$M, respectively) of the excitatory response to cholecystokinin (CCK-8, $10^{-9}$M). ACh max: acetylcholine $10^{-6}$M; ■: washout.

Fig. 4. - a, b and d: The administration of prostaglandin E$_1$ (PGE$_1$, $2.8 \times 10^{-8}$M in a, and $5 \times 10^{-9}$M in b and d) elicited a contraction of guinea-pig isolated ileum. The addition of naloxone (N, $5.5 \times 10^{-7}$M) in the declining phase caused an excitatory response. c and e: Aspirin (ASA, $5.5 \times 10^{-5}$M and $11 \times 10^{-5}$M, respectively), administered 1 min before PGE$_1$, elicited a dose-related reduction of both contractile responses. ACh max: acetylcholine $10^{-6}$M; ■: washout.
Fig. 5. - Indomethacin (IND, 2.7 x 10^{-6} M), elicited a reduction of the excitatory response to prostaglandin E\textsubscript{1} (PGE\textsubscript{1}, 5 x 10^{-9} M) and naloxone (N, 5.5 x 10^{-7} M). ACh max: acetylcholine 10^{-6} M; ■: washout.

Fig. 6. - Dose-related reduction by salicylic acid (SA, b and d, 6.2 x 10^{-5} M and 12.4 x 10^{-5} M, respectively) of the excitatory response to prostaglandin E\textsubscript{1}, (PGE\textsubscript{1}, 5 x 10^{-9} M) and naloxone (N, 5.5 x 10^{-7} M). ACh max: acetylcholine 10^{-6} M; ■: washout.
These findings do not rule out the possibility that NSAIDs act also peripherally to reduce inflammation as well as our results do not exclude a role of PGs synthesis inhibition on analgesic and anti-inflammatory effects of these drugs. The present results suggest an alternative explanation that may in part account for the effects of NSAIDs and may explain how central NSAIDs reduce inflammation. These drugs may inhibit the release of some mediator/s, like they inhibit the ACh release in the isolated ileum, somewhere in the descending neuronal pathways involved in the neurogenic aspect of inflammation, as they may inhibit the release of inflammation mediators acting on primary nociceptive afferent neurons [9]. This inhibitory effect of aspirin-like drugs appears to parallel also their central analgesic effects, although the neuronal pathways may be different and remain to be elucidated.

The tissue defense reaction to injury and irritation are associated with the local release of various mediators which include serotonin, bradykinin, histamine, substance-P, prostaglandins and other substances perhaps not all yet discovered. The action of NSAIDs on the periphery is unlike to be ascribed only to the inhibition of PGs synthesis as these do not control or modulate all endogenous substances involved in the inflammatory processes. An important component of their action may be the inhibition on the release of these endogenous substance or on their action as indicated by our results and those obtained with tiaramide. This anti-inflammatory drug which does not inhibit directly the cyclooxygenase, inhibits bradykinin-induced contraction [21]. This point of view might reasonably explain why many NSAIDs are clinically effective as analgesic and anti-inflammatory in spite of their weak or even lacking activity on PGs synthesis.

The traditional thoughts about the mechanisms of action are changing not only about NSAIDs but also with regard to opioids. It has recently been shown that, in a model of hindpaw inflammation in rat, the anti-inflammatory action of opioids involves a peripheral opioid receptor-specific mechanism of action [22-24]. It is conceivable that in inflammatory conditions the effects of algogenic substance are inhibited by opioid agonists. This mechanism is different from that of NSAIDs but the result is similar. The findings of this work that some indirectly acting substances involved in the inflammatory and algic processes such as PGE, CCK-8 and bradykinin (not shown), are able to activate the opioid system may explain why the action of endogenous opioids become apparent only under certain pathophysiological conditions [9]. The involvement of opioid system stresses that inflammation is a defense reaction to noxious stimuli consisting of a chain of physiological mechanisms of action and reaction, similarly to what observed in other phenomena [17].

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REFERENCES


