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Flavonoids Reduce Morphine Withdrawal In-vitro

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Abstract

The effects of quercetin, flavone, catechin and chrysin on the naloxone-precipitated withdrawal contracture of the acute morphine-dependent guinea-pig ileum have been investigated in-vitro.

After 4 min in-vitro exposure to morphine a strong contracture of guinea-pig isolated ileum was observed after the addition of naloxone. All the flavonoids, injected 10 min before morphine at concentrations between 10^{-7} and 10^{-5} M, were capable of blocking naloxone-induced contracture after exposure to morphine in a concentration-dependent fashion. IC50 values calculated for quercetin, flavone, catechin and chrysin were $2{\cdot}7 \times 10^{-6}$, $7{\cdot}3 \times 10^{-7}$, $8{\cdot}5 \times 10^{-7}$ and $5{\cdot}3 \times 10^{-6}$ M, respectively.

These results suggest that flavonoids might play an important role in the control of morphine withdrawal.

Flavonoids, natural compounds widely distributed in the plant kingdom, have biochemical and pharmacological activity (Middleton & Kandaswami 1993). Thus, several enzymes involved in intracellular signal transduction, for example lipoxygenase (Welton et al 1988; Laughton et al 1991), phospholipase A_2 (Lee et al 1982), membrane ATPases (Middleton & Kandaswami 1993) and a number of protein kinases including phosphatidylinositol 4-kinase (Nishioka et al 1989) and protein kinase C (Ferriola et al 1989) can be inhibited by flavonoids. Flavonoids can reduce formation of leukotriene B_4 (Middleton & Kandaswami 1993). Quercetin has been shown to down-regulate lymphocyte and natural killer-cell cyotoxicity and neutrophil function, histamine release from mast cells and adhesion-molecule expression (Middleton & Kandaswami 1993). Flavonoids have also long been recognized as antioxidative and free-radical scavenging agents (Bors et al 1990; Nakayama et al 1993; Galvéz et al 1994).

In our previous paper we demonstrated that flavonoids inhibited guinea-pig ileal muscle contractions induced by prostaglandin E_2, leukotriene D_4, acetylcholine, barium chloride and histamine, as well as electrical contractions (Capasso et al 1988a, b).

The purpose of this work was to explore further the pharmacological effects of flavonoids. Therefore, given the above evidence we decided to study the effects of some flavonoids (quercetin, flavone, catechin and chrysin) on morphine withdrawal in-vitro.

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Materials and Methods

Drugs

Naloxone.HCl, quercetin, flavone, catechin and chrysin were purchased from Sigma (St Louis, MO) and morphine.HCl from Carlo Erba (Milan, Italy).

Animals

Experiments were performed with male Charles River guinea-pigs, 180–200 g. The animals were housed in colony cages (four per cage) under conditions of standard lighting (lights on from 0700 to 1900 h), temperature (22 ± 1 °C) and humidity ($60 \pm 10\%$) for at least a week before the experiments. Food and water were freely available.

Effect of morphine withdrawal on guinea-pig ileum

The experimental procedure was that described previously (Capasso et al 1996). The ilea were left to equilibrate for 40–60 min without washing and the response to acetylcholine (10^{-6} M) was deter-

mined three times so that the response could be expressed as a percentage of the maximum response to acetylcholine. Reproducible acute opiate dependence was obtained by performing the following experimental procedure. A typical trace of contracture responses of the ileum to repeated challenges with opiate and naloxone is shown in Figure 1. After three similar acetylcholine responses, the preparation was electrically stimulated for 10–20 min (0·5-ms pulse delivered transmurally, at a frequency of 10 s at supramaximum voltage 25V). Before addition of the morphine to the bath the electrical stimulation was switched off. Under these conditions, the first contact with the opioid agonist, then, after 4-min exposure, introduction of naloxone induced a strong contraction (about 60% of the acetylcholine maximum). However, after wash-out another acetylcholine response was measured (to determine whether the ileum responsiveness was modified after withdrawal contracture (Figure 1A)) and, after 30-min resting period under stimulation, a further 4-min exposure of the ileum (without electrical stimulation) to the opiate and naloxone elicited a reproducible response. After wash-out, acetylcholine response (Figure 1B), and another 30-min resting period under stimulation, the ileum responded again to the morphine and naloxone with the same intensity (Figure 1C). In our experiments, to avoid possible tolerance to repeated morphine injection each preparation was submitted to three challenges only with morphine and naloxone. Naloxone alone did not induce effects on naive preparations or on those washed after opiate contact.

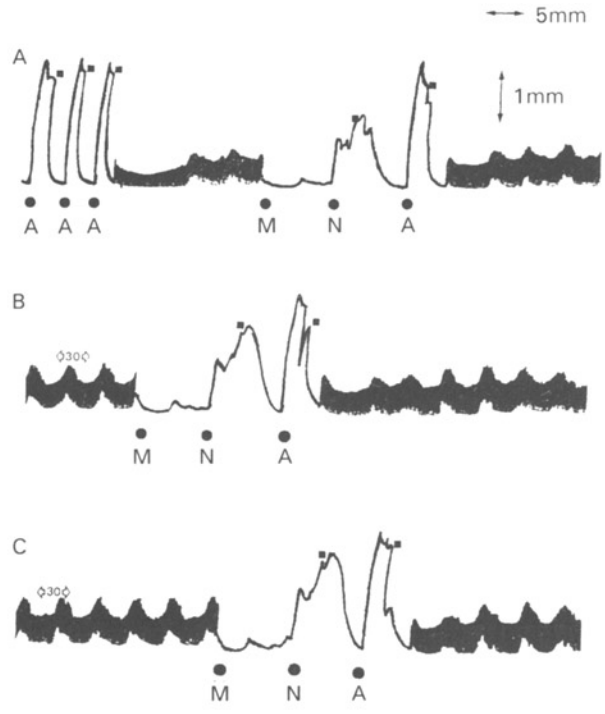

Figure 1. Typical trace of the effect of morphine withdrawal on guinea-pig ileum. A. Three similar responses to acetylcholine (A), then electrical stimulation and injection of morphine (M) are followed after 4 min by naloxone (N), which induces contraction (primary opioid withdrawal). After wash-out (■), another response to acetylcholine was elicited. B. After a 30-min resting period under electrical stimulation, a further 4-min exposure of the ileum to morphine and naloxone elicited a reproducible response (secondary opioid withdrawal). C. After another 30-min resting period under electrical stimulation, the ileum responded again to the morphine and naloxone with the same intensity (tertiary opioid withdrawal).

Determination of the effects of flavonoids

After measurement of three responses to acetylcholine and electrical stimulation for 10–20 min, morphine (10^{-5} M) was injected in the absence of electrical stimulation and then, 4 min later, naloxone (10^{-5} M) was added with subsequent contraction (primary opioid withdrawal). After wash-out, response to acetylcholine was again measured. After electrical stimulation for 30 min quercetin, flavone, catechin or chrysin (10^{-7}–10^{-5} M) were injected without electrical stimulation. Morphine was added 10 min later, then naloxone (secondary opioid withdrawal). After wash-out, response to acetylcholine was again measured. Electrical stimulation for 30 min was followed by final control opiate withdrawal (tertiary opioid withdrawal).

Because during treatment the contact period of morphine was 10 min when compared to the pre-drug period, to avoid possible influence of the contact period we performed a series of experiments to verify whether a contact period longer than 4 min affected naloxone contracture. No differences were observed when the contact period exposure of morphine was 4 or 10 min (data not shown).

Each experiment was performed on at least six to nine isolated preparations from different animals.

Parameter evaluation

Naloxone contracture. The size of the contraction induced by the naloxone challenge was expressed as a fraction of the maximum contraction obtained with the subsequent addition of acetylcholine to the same piece of tissue according to the method previously described (Capasso et al 1996):

$$\text{Tension ratio} = (\text{response to naloxone/maximum response to acetylcholine}) \times 100$$

Acetylcholine response before and after treatment. Reduction or increase of the acetylcholine response in the post-drug period was expressed as a percentage of acetylcholine response in the pre-drug period.

Electrically stimulated contraction before and after treatment. Reduction or increase of the electrically stimulated contraction in the post-drug period was expressed as a percentage of the electrical stimulation in the pre-drug period.

Naloxone contraction before and after treatment. Reduction or increase of the naloxone contraction in the post-drug period was expressed as a percentage of the naloxone contraction in the pre-drug period.

Statistical analysis

Results were tested for statistical significance by use of Student's *t*-test for paired data when comparing results obtained before and after treatment of the same preparation.

Results and Discussion

Under the experimental conditions used quercetin, flavone, catechin and chrysin administered 10 min before the injection of morphine resulted in dose-dependent reduction of morphine withdrawal. After wash-out acetylcholine response, electrical stimulation and final opiate withdrawal were still reduced (Table 1).

These results indicate that flavonoids have a significant effect on opiate withdrawal in-vitro, suggesting an important involvement of flavonoids in the control of opioid withdrawal. The literature contains no information about the effect of flavonoids on morphine withdrawal and this is the first paper reporting this property of flavonoids.

Although the mechanisms by which flavonoids control opiate withdrawal are still unclear, several possibilities should be considered. It has been demonstrated that acetylcholine plays an important role in the expression of opiate withdrawal because cholinergic agonists exacerbate opioid withdrawal whereas muscarinic and nicotinic blockers attenuate some aspects of the syndrome (Martin & Eades 1967; Bhargava & Way 1972). Furthermore, a large proportion of the contracture in opioid withdrawal in-vitro is a result of acetylcholine release because it is blocked by atropine or hyoscine (Tsou et al 1982; Chal 1983).

A relationship between the cholinergic system and flavonoids has been reported (Capasso et al 1988a) because flavonoids inhibit contractions induced by acetylcholine in guinea-pig isolated ileum. Therefore, one might suggest that the effects observed might be related to alteration of cholinergic neuron activity and the ability of flavonoids to reduce morphine withdrawal might be related to their anticholinergic activity. Our results strongly support this hypothesis because the effects of both acetylcholine and electrical stimulation were still reduced after wash-out of flavonoids, indicative of the direct action of flavonoids both on post- and pre-synaptic acetylcholine receptors.

Flavonoids have been reported to have good anti-inflammatory activity because of their capacity to inhibit formation of arachidonic acid metabolites (Lee et al 1982; Welton et al 1988; Laughton et al 1991; Middleton & Kandaswami 1993). We recently demonstrated that arachidonic acid metabolites are involved in the expression of opiate withdrawal because inhibitors of phospholipase A_2, cyclooxygenase and 5-lipoxygenase reduce opiate dependence (Capasso & Sorrentino 1997). Therefore, in our experiments, we cannot exclude the possibility that reduction of morphine withdrawal by flavonoids might be related to their inhibiting activity on arachidonic acid metabolites.

In conclusion, our data indicate that flavonoids exert important control on morphine withdrawal phenomena. Further studies are necessary to better elucidate the mechanism underlying this interaction.

Table 1. The effect of quercetin, flavone, catechin and chrysin (10^{-7}–10^{-5} M) on morphine withdrawal (naloxone contraction), acetylcholine contraction, electrical stimulation contraction and final naloxone contraction.

Substance	Naloxone contraction (M)	Acetylcholine contraction (M)	Electrical stimulation contraction (M)	Final naloxone contraction (M)
Quercetin	$2{\cdot}7 \times 10^{-6}$ ($1{\cdot}4 \times 10^{-7}$–$3{\cdot}7 \times 10^{-5}$)	$1{\cdot}5 \times 10^{-7}$ ($3{\cdot}8 \times 10^{-8}$–$3{\cdot}7 \times 10^{-6}$)	$4{\cdot}2 \times 10^{-7}$ ($2{\cdot}5 \times 10^{-8}$–$7{\cdot}1 \times 10^{-6}$)	$5{\cdot}6 \times 10^{-7}$ ($3{\cdot}8 \times 10^{-8}$–$4{\cdot}1 \times 10^{-6}$)
Flavone	$7{\cdot}3 \times 10^{-7}$ ($3{\cdot}7 \times 10^{-8}$–$4{\cdot}9 \times 10^{-6}$)	$5{\cdot}7 \times 10^{-8}$ ($4{\cdot}2 \times 10^{-9}$–$1{\cdot}7 \times 10^{-7}$)	$6{\cdot}2 \times 10^{-8}$ ($5{\cdot}1 \times 10^{-9}$–$8{\cdot}3 \times 10^{-7}$)	$3{\cdot}8 \times 10^{-8}$ ($1{\cdot}6 \times 10^{-9}$–$2{\cdot}7 \times 10^{-7}$)
Catechin	$8{\cdot}5 \times 10^{-7}$ ($1{\cdot}7 \times 10^{-8}$–$2{\cdot}6 \times 10^{-6}$)	$2{\cdot}4 \times 10^{-7}$ ($5{\cdot}3 \times 10^{-8}$–$7{\cdot}1 \times 10^{-6}$)	$1{\cdot}5 \times 10^{-7}$ ($2{\cdot}4 \times 10^{-8}$–$6{\cdot}3 \times 10^{-6}$)	$3{\cdot}2 \times 10^{-7}$ ($6{\cdot}4 \times 10^{-8}$–$8{\cdot}2 \times 10^{-6}$)
Chrysin	$5{\cdot}3 \times 10^{-6}$ ($4{\cdot}1 \times 10^{-7}$–$6{\cdot}5 \times 10^{-5}$)	$3{\cdot}2 \times 10^{-6}$ ($1{\cdot}4 \times 10^{-7}$–$4{\cdot}6 \times 10^{-5}$)	$4{\cdot}7 \times 10^{-6}$ ($3{\cdot}6 \times 10^{-7}$–$5{\cdot}2 \times 10^{-5}$)	$2{\cdot}4 \times 10^{-6}$ ($7{\cdot}3 \times 10^{-7}$–$4{\cdot}2 \times 10^{-5}$)

Results are expressed as IC50 with confidence limits. Each substance was injected 10 min before morphine.

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