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Short communication

Involvement of calcium in flavonoid analgesia

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Calcium has been reported to play a key role in opioid analgesia. Several flavonoids also elicit an analgesic effect through the opioid system. The involvement of calcium in the analgesic activity of these flavonoid compounds was now investigated. Calcium antagonised the analgesic action of flavonoids while nifedipine, a calcium channel blocker, potentiated it. This suggests a possible role for calcium in the analgesic action of flavonoids as with that of morphine.

Flavonoids; Ca^{2+}; Analgesia

1. Introduction

The biological significance of flavonoids is now widely recognised. Experiments conducted in our laboratory have revealed a significant analgesic activity for some flavonoid compounds (Viswanathan et al., 1984; Thirugnanasambantham et al., 1985). As an extension of this work, a series of flavonoid substances were tested for their analgesic activity. An attempt was made to investigate the possible structure-activity relationship with respect to analgesia. Flavone and its hydroxy derivatives were synthesised in our laboratory and were screened. While all the flavonoid substances tested elicited analgesia, it was interesting that some of these involved opioid pathways to produce their analgesic action (unpublished observation).

It has been demonstrated that the divalent cation, calcium, exerts a physiological role in the control of pain either directly, or indirectly through endogenous analgesic agents. Exogenous calcium was found to induce hyperalgesia as well as to antagonise the analgesic effect of opioids whereas calcium chelators potentiated this effect (Harris et al., 1975). Since some of the flavone derivatives involved opioid mediation in their analgesic action, the present experiments were designed to investigate the role of calcium in the analgesic activity of these derivatives.

2. Materials and methods

Male albino mice (20-25 g) were chosen for the study. Analgesia was assessed by means of acetic acid-induced writhing (Koster et al., 1959). The writhings after i.p. injection of acetic acid (0.6%; 10 ml/kg) were counted for 15 min. Injections of flavonoids (suspension in 1% carboxymethyl cellulose) or morphine sulfate (0.25 mg/kg) were given s.c. 60 min or 30 min prior to acetic acid challenge respectively. The analgesic and sub-analgesic doses of flavonoids were selected from

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our earlier experiments on analgesia. Some of the animals received calcium (1.72 mEq/kg i.v.) or nifedipine (40 μg/kg i.v.) 10 min prior to the acetic acid challenge and analgesia was assessed as described above. The effect of calcium or nifedipine themselves on acetic acid writhing was studied in separate groups of mice.

2.1. Drugs and chemicals

Flavone (Org. Syntheses, 1952, 32, 72; the 5-hydroxy- (Looker et al., 1964), 7-hydroxy- (Baker, 1933), 2′-hydroxy (Baker and Besly, 1940), 5,7-dihydroxy- (Rama Rao et al., 1964) and 7,8-dihydroxy- (Baker, 1933) flavones synthesised in our laboratory; morphine sulfate (Government Opium Alkaloids Works, Ghazipur) and acetic acid (BDH). The results were analysed with Dunnett's test.

3. Results

Morphine and flavone and its hydroxy derivatives produced a significant reduction in the number of writhings (table 1). Calcium or nifedipine had no effect on acetic acid-induced writhing. Neither morphine nor flavone and its derivatives could produce any inhibitory effect on writhing in calcium-treated animals. Sub-analgesic doses of morphine, flavone or its hydroxy derivatives produced no significant inhibition of writhing. A significant reduction in the number of writhings was observed when nifedipine was combined with the same doses of the above drugs (table 2).

TABLE 1

Effect of calcium on flavonoid and morphine analgesia. Acetic acid was administered 60 min after the flavonoid or 30 min after morphine. Calcium was administered 10 min prior to acetic acid challenge. Each value represents the mean number of writhings ± S.E.M. from a minimum of six observations.

First treatment (mg/kg s.c.)	Second treatment	
	Saline	Calcium (1.7 mEq/kg i.v.)
Vehicle	31.3 ± 2.5	30.5 ± 2.0
Morphine (0.25)	15.5 ± 0.3 [b]	23.8 ± 1.0 [a]
Flavone (60)	15.5 ± 1.6 [b]	21.6 ± 1.6 [a]
5-Hydroxyflavone (40)	14.2 ± 1.7 [b]	24.6 ± 1.8 [a]
7-Hydroxyflavone (125)	16.3 ± 2.6 [b]	26.1 ± 1.1 [a]
2′-Hydroxyflavone (100)	13.6 ± 1.8 [b]	21.3 ± 1.9 [a]
5,7-Dihydroxyflavone (40)	14.8 ± 1.9 [b]	26.7 ± 1.1 [a]
7,8-Dihydroxyflavone (200)	17.3 ± 0.8 [b]	24.2 ± 2.6 [a]

[a] $P < 0.05$ as compared with saline treatment. [b] $P < 0.05$ as compared with vehicle treatment.

TABLE 2

Effect of nifedipine on flavonoid and morphine analgesia. Acetic acid was injected 60 min after the flavonoid or 30 min after morphine. Nifedipine was administered 10 min prior to acetic acid challenge. Each value represents the mean number of writhings ± S.E.M. from a minimum of six observations.

First treatment (mg/kg s.c.)	Second treatment	
	Saline	Nifedipine (40 μg/kg i.v.)
Vehicle	32.0 ± 1.1	31.1 ± 2.3
Morphine (0.05)	24.5 ± 1.2	14.8 ± 1.5 [a]
Flavone (5)	28.3 ± 2.0	19.1 ± 1.2 [a]
5-Hydroxy flavone (5)	26.5 ± 1.1	17.3 ± 1.7 [a]
7-Hydroxy flavone (10)	28.4 ± 1.5	18.0 ± 2.1 [a]
2′-Hydroxy flavone (10)	27.8 ± 2.1	19.8 ± 1.8 [a]
5,7-Dihydroxy flavone (5)	26.5 ± 0.8	17.0 ± 1.2 [a]
7,8-Dihydroxy flavone (50)	29.3 ± 1.3	18.5 ± 1.5 [a]

[a] $P < 0.05$ as compared with saline treatment.

4. Discussion

Morphine, flavone and its hydroxy derivatives exhibited, as observed in earlier studies, a significant analgesic effect as evidenced by a reduced number of writhings. The present data showed clearly that exogenous calcium antagonises the analgesic action of morphine as well as that of flavone and its derivatives, while nifedipine potentiates the analgesic response of the above compounds.

This observation suggests that calcium plays a role in the analgesic action of flavonoids, possibly similar to its role in morphine analgesia. This observation is in agreement with the earlier report describing a similar role for calcium in gossypin-induced analgesia, which is mediated through the opioid system (Viswanathan et al., 1984; 1985). It can be proposed, based on previous findings and on the present results, that the analgesic effect of

flavonoids resembles that of morphine and that a common mechanism could be operating in their analgesic action.

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