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Role of calcium in the analgesic action of tetrahydroisoxazolepyridinol and muscimol

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Abstract—The role of calcium in the analgesic activity of 4,5,6,7-tetrahydroisoxazole-(5,4-c) pyridin-3-ol (THIP) and muscimol has been investigated using the acetic acid assay procedure. THIP and muscimol produced significant analgesic response. Whilst neither calcium chloride nor nifedipine in the doses employed significantly altered the number of writhings, calcium chloride antagonized the analgesic responses of THIP and muscimol while nifedipine potentiated them. Similar responses were recorded for morphine. These findings suggest that THIP and muscimol, like morphine, might alter the calcium movements across the plasma membrane to elicit the analgesic response.

Recent studies indicate that alterations either in the transport of calcium ions across the plasma membranes or in brain calcium metabolism might play a significant role in the production of analgesia (Cardenas & Ross 1976; Harris et al 1977; Yamamoto et al 1978). The same was found to be true for other non-opioid analgesic agents acting through opioid mechanisms (Ramaswamy et al 1986a, b). It was reported that exogenous calcium given intracerebroventricularly antagonized morphine analgesia while the calcium chelator ethylene glutamine tetraacetic acid (EGTA) potentiated it (Ross 1977; Schmidt & Way 1980). Analgesic agents like thyrotropin releasing hormone (TRH) and clonidine, which act through non-opioid mechanisms, do not involve calcium in their action. Thus, it was suggested that the effect of calcium could be used as a parameter for differentiating the agents acting through opioid and non-opioid mechanisms (Ramaswamy et al 1986a).

GABA agonists, 4,5,6,7-tetrahydroisoxazole-(5,4-c) pyridin-3-ol (THIP) and muscimol, produced analgesia (Hill et al 1981; Andree et al 1983). Recently, using a sensitive chemical assay model, it has been demonstrated that THIP and muscimol utilize opioid pathways in eliciting analgesia (Srinivasan et al 1990). In the present study, the role of calcium in the analgesic action of THIP and muscimol was explored.

Materials and methods

Swiss male albino mice, 20–25 g, were housed with a 12 h dark:12 h light cycle for at least two days before experimentation, and had free access to food and water.

Analgesia was assessed by injecting acetic acid (0·6%, 10 mL kg^{-1}) intraperitoneally. The number of writhings produced during the following 10 min and the time at which the animal started writhing (onset) were recorded. A significant reduction in the number of writhings and a delay in the onset compared with saline-treated animals were considered an analgesic response. Each animal was subjected to an analgesic test once only.

Treatment. The control animals received 0·9% NaCl (saline). Separate groups of animals received morphine (0·05 mg kg^{-1}, i.p.), THIP (0·05 mg kg^{-1}, i.p.) or muscimol (0·1 mg kg^{-1}, i.p.) and, 15 min after, the acetic acid challenge was made. Different groups of animals were treated with either nifedipine (40 mg kg^{-1}, i.v.) or calcium chloride (0·86 mmol kg^{-1}) alone or 10 min after THIP, muscimol or morphine; the acetic acid challenge was made 5 min later.

The results were analysed by ANOVA followed by Dunnett's *t*-test.

Drugs used were: calcium chloride (A. R. Sarabhai), THIP bitartrate (Sigma), morphine hydrochloride (Government Alkaloid Works, Ghazipur, India), muscimol (Sigma) and nifedipine (Sigma).

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Results

Morphine, THIP or muscimol significantly reduced the number of writhings and delayed their onset (Table 1). Neither nifedipine nor calcium chloride modified delay or onset significantly. However, morphine, THIP or muscimol were unable to produce any significant reduction in the number of writhings or delay their onset in animals subsequently treated with calcium chloride, but their inhibitory effect on writhing and ability to delay its onset were significantly enhanced in animals given nifedipine (Table 1).

Discussion

It has been documented that GABA mimetics, THIP and muscimol, produce a potent analgesic response when tested by various assays (Hill et al 1981; Andree et al 1983). However, their mechanism is unclear. Even though development of a chronic tolerance and presence of a cross-tolerance with morphine have been demonstrated, a direct role for the involvement of the opioid system in this action could not be ascertained (Hill et al 1981; Andree et al 1983). In a recent review (Sivam & Ho 1985), it was suggested that the choice of analgesic assay could be an important variable for the observed conflicting results regarding the mechanism of GABA mimetic analgesia. Considering this, the role of the opioid system was re-examined (Srinivasan et al 1990) using the acetic acid assay which has been suggested to be the most sensitive (Hayashi & Takemori 1981). In the study of Srinivasa et al (1990), a consistent role for the opioid system in the analgesic action of THIP and muscimol was recorded—since naloxone reversed their analgesic action—and acute and chronic tolerance developed to it as for morphine (Srinivasan 1989).

The results of the present study further support the possibility of morphine and GABA mimetics acting through a common mechanism for producing analgesia. Like morphine, the analgesic response of THIP and muscimol was potentiated by nifedipine and antagonized by calcium chloride, suggesting that THIP and muscimol might alter calcium ion movements across the plasma membrane to elicit analgesia. Clonidine and TRH which utilize pathways other than the opioid system did not involve calcium in their analgesic response (Ramaswamy et al 1986a). Hence, it appears that analgesics acting through the opioid system involve calcium at the cellular level.

The present study along with earlier reports (Srinivasan 1989; Srinivasan et al 1990) suggests a close interaction between the GABA and opioid systems in the genesis of analgesia.

Table 1. The influence of calcium chloride and nifedipine on the analgesic effect of morphine, THIP and muscimol in mice.

First treatment (mg kg^{-1})	Second treatment	Number of writhings	Onset (s)
Saline	Saline	25·5 ± 1·3	211·7 ± 15·6
Morphine (0.05)	Saline	14·9 ± 1·9[a]	318·0 ± 9·4[a]
THIP (0·05)	Saline	13·3 ± 0·9[a]	315·0 ± 18·6[a]
Muscimol (0·1)	Saline	13·7 ± 1·0[a]	290·0 ± 10·0[a]
Saline	$CaCl_2$ (0·86 mmol kg^{-1})	23·4 ± 3·4	200·0 ± 12·0
Saline	Nifedipine (40 μg kg^{-1})	24·2 ± 1·2	220·0 ± 9·4
Morphine (0·05)	$CaCl_2$ (0·86 mmol kg^{-1})	23·8 ± 3·2[b]	218·0 ± 11·2[b]
THIP (0·05)	$CaCl_2$ (0·86 mmol kg^{-1})	22·8 ± 1·7[b]	200·0 ± 10·0[b]
Muscimol (0·1)	$CaCl_2$ (0·86 mmol kg^{-1})	19·3 ± 0·9[b]	200·0 ± 14·8[b]
Morphine (0·05)	Nifedipine (40 μg kg^{-1})	4·8 ± 1·1[b]	340·0 ± 10·0[b]
THIP (0·05)	Nifedipine (40 μg kg^{-1})	9·8 ± 0·9[b]	360·0 ± 7·7[b]
Muscimol (0·1)	Nifedipine (40 μg kg^{-1})	9·0 ± 0·6[b]	350·0 ± 6·3[b]

Each value represents the mean ± s.e.m. of 6 experiments. [a]$P < 0·05$ compared with saline-saline value. [b]$P < 0·05$ compared with the value of their respective drug-saline treatment values.

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