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A STUDY ON THE ROLE OF CHOLINERGIC AND GAMMA AMINO BUTYRIC ACID SYSTEMS IN THE ANTI-NOCICEPTIVE EFFECT OF GOSSYPIN

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SUMMARY

1. The participation of cholinergic and gamma amino butyric acid (GABA) neurotransmitter systems in the anti-nociceptive effect of gossypin was investigated using pharmacological tools.

2. Physostigmine potentiated its anti-nociceptive response while atropine failed to modify it significantly.

3. THIP (4,5,6,7-tetra hydroisoxazolo (5,4-C) pyridin-3-ol) and gossypin treatment produced an additive response while bicuculline attenuated the anti-nociceptive response of gossypin. Similar observations were recorded for morphine.

4. It is suggested that cholinergic and GABAergic systems play a role in gossypin-induced anti-nociception.

Key words: anti-nociception, cholinergic, GABA, gossypin, morphine.

INTRODUCTION

Gossypin, a bioflavonoid, has been shown to exhibit opioid mediated anti-nociceptive activity which was found to be less potent (500×) than morphine as tested in the abdominal constriction test. The type of opiate receptor involved may be the same as that which morphine acts on (apparent pA_2 values are near identical). This is supported by the observation that naloxone reverses the anti-nociceptive action of morphine and gossypin almost equally (Viswanathan *et al.* 1984). As with morphine, gossypin seems to alter the transport of calcium ions across the cell membranes to elicit anti-nociception (Viswanathan *et al.* 1985). In contrast to morphine, tolerance to and dependence on gossypin did not develop, suggesting that this flavonoid substance may have a therapeutic advantage over morphine (Viswanathan *et al.* 1985, 1990).

A role for multi-neurotransmitter systems involving cholinergic, serotonergic and GABAergic systems etcetera in the morphine-induced anti-nociception has been reported (Samanin & Valzelli 1971; Yoneda *et al.* 1976; Koehn & Karczmar 1978). However, such participation has not been examined for gossypin except the observation that α_2-adrenergic receptors do not contribute to its anti-nociceptive activity (Viswanathan 1984). Since gossypin resembles morphine in its anti-nociceptive activity in some respects, an attempt has been made in the present study to examine the role of cholinergic and GABAergic systems in gossypin-induced anti-nociception using pharmacological manoeuvres. Cholinergic function was increased by physostigmine and decreased by atropine, while such alterations in GABAergic functions were achieved by

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THIP (4,5,6,7-tetra hydroisoxazolo (5,4-C) pyridin-3-ol) and bicuculline, respectively.

METHODS

Male Swiss albino mice (20–25 g) housed in our animal house at room temperature (25 ± 2°C) with food and water *ad libitum* were used.

Anti-nociceptive effect was assessed using acetic acid-induced writhing assay (Koster *et al.* 1959). The number of writhings for 10 min after the injection of acetic acid (0.6%; 10 mL/kg, i.p.) were counted. Any significant reduction in the number of writhings as compared with saline treated animals was considered an anti-nociceptive response. It is expressed as per cent inhibition taking the number of writhings in saline treated animals as 100%.

The effect of morphine (0.1 mg/kg, i.p.), THIP (0.01 mg/kg, i.p.) and gossypin (50 mg/kg, i.p.) on acetic acid-induced writhing response was recorded by treating the animals with the above agents 15, 30 or 60 min, respectively, prior to acetic acid challenge. The doses of morphine and gossypin used are the approximate ED_{50} doses. Additionally, their effect was recorded in animals exposed to physostigmine (0.01 mg/kg, i.p.), atropine sulfate (1.0 mg/kg, i.p.) or bicuculline (2.0 mg/kg, i.p.) 15 min prior to the anti-nociceptive agents. The effect of THIP (0.01 mg/kg, i.p.) in animals pretreated with gossypin (50 mg; 60 min prior to THIP) or morphine (0.1 mg/kg; 15 min prior to THIP) was also noted. Since THIP is short-acting it was administered later than morphine or gossypin in these studies. The influence of the interacting agents viz. physostigmine, atropine and bicuculline in the doses employed on the acetic acid-induced writhing was studied in an independent group of animals. The time intervals for pretreatments and treatments were chosen from our earlier studies (Viswanathan *et al.* 1984; Ramaswamy *et al.* 1989). Drugs were dissolved in saline except bicuculline which was dissolved in acidified saline (0.1 mol/L HCl; 0.1 mL). Saline treated animals served as controls. The data were subjected to ANOVA followed by Dunnett's *t*-test for statistical analysis.

Drugs administered

The drugs used were acetic acid, glacial (AR, Sarabhai, Vadodra, India), atropine sulfate and (+) bicuculline (Sigma, St Louis, MO, USA). Gossypin was extracted in our laboratory from *Hibiscus vitifolius* flowers according to the method of Rao and Seshadri (1946). The identity and purity of the compound were tested by a comparison of the melting point, thin layer chromatography and UV spectra with that of an authentic sample. Morphine sulfate (Govt Opium and Alkaloid Works, Ghazipur, India), physostigmine salicylate (Boehringer-Ingelheim) and THIP bitartrate (Sigma) were used.

RESULTS

Morphine (0.1 mg) and gossypin (50 mg) produced a significant reduction in the number of writhings while THIP (0.1 mg) produced an insignificant decrease (Table 1). Physostigmine (0.01 mg), atropine sulfate (1 mg) and bicuculline (2 mg) *per se* did not modify the number of writhings significantly. Physostigmine pretreatment significantly enhanced the inhibitory effect of morphine as well as gossypin to a great extent, whereas prior exposure to atropine failed to significantly modify such a response (Table 1). THIP (0.01 mg) *per se* had no pronounced effect on writhing response. However, at this dose it was found to produce an additive response with morphine (0.1 mg) or gossypin (50 mg) treatment. Bicuculline (2 mg) pretreatment attenuated the inhibitory effect of morphine and gossypin (Table 1).

DISCUSSION

Several neurotransmitter systems, apart from exhibiting inherent anti-nociceptive activity, have been shown to make a significant contribution in morphine-induced anti-nociception. For example, cholinergic and GABAergic agents induce a potent anti-nociceptive response (Pedigo *et al.* 1975; Hill *et al.* 1981). Further, it has been established that morphine alters the functions of these neurotransmitter systems to elicit anti-nociception. (Jhamandas & Sutak 1974; Yoneda *et al.* 1976; Grognet *et al.* 1983). Prolactin, an endocrine peptide, produced an opioid mediated anti-nociceptive response (Ramaswamy *et al.* 1983). A role for cholinergic and GABAergic systems in the action of prolactin has been noted (Ramaswamy *et al.* 1986, 1989).

Gossypin-induced anti-nociception resembled that induced by morphine, as it was attenuated by naloxone and the pA_2 values for naloxone against gossypin are similar to those recorded for morphine (Viswanathan *et al.* 1984). These observations substantiate opioid modulation of gossypin-induced anti-nociception. The observation of this present study that physostigmine *per se* in subeffective doses potentiates gossypin anti-nociception, in a similar manner to morphine, indicates an involvement for cholinergic systems in this effect

Table 1. The influence of cholinergic and GABAergic agents on the changes induced by morphine and gossypin on the acetic acid-induced writhing in mice

Pretreatment (mg/kg)	Treatment (mg/kg)	No. writhings	% inhibition
Saline	Saline	23.7 ± 0.9	—
Saline	Gossypin 50.0	12.3 ± 0.7*	48.2
Saline	Morphine 0.1	11.8 ± 1.2*	50.3
Physostigmine 0.01	Saline	21.9 ± 1.1	10.2
Atropine 1.0	Saline	22.4 ± 0.8	5.5
Physostigmine 0.01	Morphine 0.1	4.8 ± 0.4*†	79.2
Physostigmine 0.01	Gossypin 50.0	3.4 ± 0.7*†	85.7
Atropine 1.0	Morphine 0.1	13.7 ± 0.9*	42.8
Atropine 1.0	Gossypin 50.0	15.9 ± 0.4*	33.0
Saline	THIP 0.01	19.7 ± 0.9	16.9
Bicuculline 2.0	Saline	20.7 ± 1.2	12.7
Morphine 0.1	THIP 0.01	7.8 ± 0.7*†	67.1
Gossypin 50.0	THIP 0.01	6.3 ± 0.5*†	73.5
Bicuculline 2.0	Morphine 0.1	18.4 ± 0.8*†	22.4
Bicuculline 2.0	Gossypin 50.0	17.3 ± 0.6*†	27.1

All values mean ± s.e.m. of six experiments.
*$P<0.01$ as compared with saline-saline value (t-test; 10 d.f.).
†$P<0.05$ as compared with their respective saline pretreatment value (t-test; 10 d.f.).

of gossypin. However, atropine failed to attenuate the anti-nociceptive effect of gossypin significantly. A similar response was observed for morphine in this, and an earlier study (Koehn & Karczmar 1976), and also for prolactin (Ramaswamy *et al.* 1986). This particular aspect is difficult to explain with the available data and deserves detailed investigation.

The additive response (recorded with THIP; subthreshold dose) of the gossypin-induced anti-nociception and the antagonism of the same by bicuculline strongly suggest that the GABAergic transmitter system contributes to this action of gossypin, as has been observed for morphine.

The findings of the present study suggest that multi-neurotransmitter pathways might be involved in gossypin-induced anti-nociception. While a role for cholinergic and GABAergic systems were indicated in this investigation, further studies on the involvement of other neurotransmitter systems would be worthwhile.

REFERENCES

Grognet, A., Hertz, F. & Defeudis, F. V. (1983) Comparison of the analgesic actions of THIP and morphine. *General Pharmacology*, **14**, 585–589.

Hill, R. C., Maure, R., Buescher, H. H. & Roemer, D. (1981) Analgesic properties of the GABA mimetic THIP. *European Journal of Pharmacology*, **69**, 221–224.

Jhamandas, K. & Sutak, M. (1974) Modification of brain acetylcholine release by morphine and its antagonists in normal and morphine dependent rats. *British Journal of Pharmacology*, **50**, 57–67.

Koehn, G. L. & Karczmar, A. G. (1978) Effect of diisopropyl phosphofluridate on analgesia and motor behavior in the rat. *Progress in Neuro-Psychopharmacology*, **2**, 169–177.

Koster, R. M., Anderson, M. & DeBeer, A. J. (1959) Acetic acid for analgesic screening. *Federation Proceedings*, **18**, 412.

Pedigo, N. W., Deway, W. L. & Harris, L. S. (1975) Determination and characterization of the anti-nociceptive activity of intraventricularly administered acetylcholine in mice. *Journal of Pharmacology and Experimental Therapeutics*, **193**, 845–852.

Ramaswamy, S., Pillai, N. P. & Bapna, J. S. (1983) Analgesic effect of prolactin: Possible mechanism of action. *European Journal of Pharmacology*, **96**, 171–173.

Ramawamy, S., Rajasekaran, M., Madusudhana Rao, K. & Bapna, J. S. (1986) The influence of cholinergic agents on the analgesic activity of prolactin and metaclopramide. *IRCS Medical Sciences*, **14**, 994.

Ramaswamy, S., Suthakaran, C. & Bapna, J. S. (1989) Role of GABAergic system in prolactin analgesia. *Clinical and Experimental Pharmacology and Physiology*, **16**, 893–896.

Rao, K. V. & Seshadri, T. R. (1946) Colouring matters of the flowers of *Hibiscus vitifolius*. *Proceedings of the Indian Academy of Sciences*, **24**, 352–356.

Samanin, R. & Valzelli, L. (1971) Increase of morphine-

induced analgesia by stimulation of the nucleus raphae dorsalis. *European Journal of Pharmacology*, **16**, 298–302.

Viswanathan, S. (1984) Pharmacological investigation on certain bioflavonoids. PhD thesis, University of Madras, Madras.

Viswanathan, S., Ramaswamy, S., Thirugnanasambantham, P., Ramachandran, S. & Kameswaran, L. (1990) Possible development of dependence on gossypin: Role of gossypin on morphine dependence. *Indian Journal of Experimental Biology*, **28**, 1193–1194.

Viswanathan, S., Thirugnanasambantham, P., Kannappa Reddy, M. & Kameswaran, L. (1984) Gossypin induced analgesia in mice. *European Journal of Pharmacology*, **98**, 289–291.

Viswanathan, S., Thirugnanasambantham, P., Ramaswamy, S. & Kameswaran, L. (1985) Studies on possible tolerance and mechanism of gossypin analgesia. *Indian Journal of Experimental Biology*, **23**, 525–526.

Yoneda, Y., Takashima, S. & Kuriyama, K. (1976) Possible involvement of GABA in morphine analgesia. *Biochemical Pharmacology*, **25**, 2669–2670.