ANTINOCICEPTIVE EFFECTS OF MORPHINE AND PHYSOSTIGMINE IN DIFFERENT INBRED STRAINS OF MICE

(Received 20 April, 1995)

A.N. Aslan, M.D.* / K. Berkman, M.D.***
B. Çırakoğlu, Ph.D. / Ş. Oktay, M.D., Ph.D.***

* *Professor, Department of Pharmacology, Faculty of Medicine, Marmara University, İstanbul, Turkey.*
** *Professor, Department of Medical Biology, Faculty of Medicine, Marmara University, İstanbul, Turkey.*
*** *Resident, Department of Pharmacology, Faculty of Medicine, Marmara University, İstanbul, Turkey.*

ABSTRACT

Antinociceptive effects of morphine and physostigmine, alone or in combination were investigated in mice of BALB/C, C57BL/6 and CB6F1 strains. Morphine and physostigmine increased pain treshold in BALB/C and CB6F1 mice at a dose of 5 mg/kg and 0.1 mg/kg, respectively, whereas C57BL/6 mice were found to be less responsive to both agents. When a nonanalgesic dose of physostigmine (0.05 mg/kg) was combined with a nonanalgesic dose of morphine (2.5 mg/kg), a significant antinociceptive effect was observed in BALB/C and CB6F1 mice, but not in C57BL/6 strain. These results suggest that there is a significant influence of strain on cholinergic and opioid antinociception, and synergistic interaction between cholinergic and opioidergic systems also occur strain-dependently.

Key Words: Analgesia, tail-flick, morphine, physostigmine, BALB/C, C57BL/6, CB6F1.

INTRODUCTION

There are several endogenous neurotransmitter systems that modulate the responses to the noxious stimuli. There is vast literature describing the antinociceptive effect of physostigmine which is a centrally acting cholinesterase inhibitor (1, 2). Along with physostigmine, other cholinomimetics are also capable of altering the nociceptive state via central muscarinic receptors (3, 4).

Cholinergic mechanisms interact with other neurotransmitter systems involved in the noxious transmission such as serotonergic, adrenergic and opioidergic systems, at any level of the central nervous system (5-8). Antinociceptive effect of intrathecal morphine was reported to be potentiated by physostigmine and attenuated by atropine (5). On the other hand, microinjections of atropine into the lateral reticular nucleus was found to facilitate the antinociceptive effect of morphine injected into the same site (8).

Mice are very frequently being used to study both nociception and antinociceptive effects of various pharmacological agents. However, it was recently reported that a special inbred strain of mice (C57BL/6) was more resistant to the analgesic effect of a muscarinic receptor agonist oxotremorine than DBA mice (9). Therefore, it seemed interesting to investigate the cholinergic influence on morphine-induced analgesia in three different inbred strains of mice.

MATERIALS AND METHODS

Male naive mice (20 - 40 g) belonging to the albino BALB/C, C57BL/6, ad CB6F1 (cross of BALB/C and C57BL/6) strains (Turkish Scientific and Technical Research Council Lab., Gebze, Istanbul) were used. Food and water were available ad libitum.

Analgesia was determined by the tail flick analgesiameter (Harvard). The intensity of the heat stimulus in the tail flick test was adjusted so that the animal flicked its tail in 2.5 - 4.0 sec. The cutoff time was set at 10 sec. Tail flick latencies (TFL) were measured before and 15, 30 and 45 min after the drug injections. The inhibition of the tail flick response was expressed as percent maximum possible effect (%MPE) using the following equation: %MPE = (postdrug latency - baseline latency) / (cutoff time - baseline latency). Animals were used only once in all the experiments. Groups of 10 - 12 animals for each strain were tested for each dose of the drugs. Morphine (2.5 and 5 mg/kg; TMO, Turkey) or physostigmine (0.05 and 0.1 mg/kg; Sigma) was

injected intraperitoneally (i.p.) in a volume of 0.1 ml/10 g. Morphine (2.5 mg/kg) and physostigmine (0.05 mg/kg) were given as seperate injections concomitantly to investigate their interaction. Saline (0.1 ml/10 g) - injected group was used as control. Results were expressed as mean ± S.E.M. An analysis of variance (ANOVA) was used to determine the significance of differences between means for comparison of morphine's and physostigmine's effects between strains. The level of significance was calculated by Dunnett's test and a level of probability of 0.05 was accepted as statistically significant.

RESULTS

Saline alone did not cause any antinociceptive effect in any of the strains (data not shown). The administration of 5 mg/kg morphine and 0.1 mg/kg physostigmine induced strain-dependent analgesic effect (Figs. 1,2). C57BL/6 mice were found to be less responsive to morphine, and physostigmine was totally ineffective (Figs. 1, 2). Physostigmine and morphine were not analgesic in all three strains at 0.05 mg/kg and 2.5 mg/kg, respectively (data not shown). When the two drugs were combined at their nonanalgesic doses, the significant antinociceptive effect observed in BALB/C and CB6F1 mice were similar in magnitude. Although the combination caused slight delayed analgesia in C57BL/6 strain, the maximum possible effects were significantly lower than the other strains at 15 and 30 minutes after the injection and were not significantly different than morphine 2.5 mg/kg alone at all times (Fig. 3).

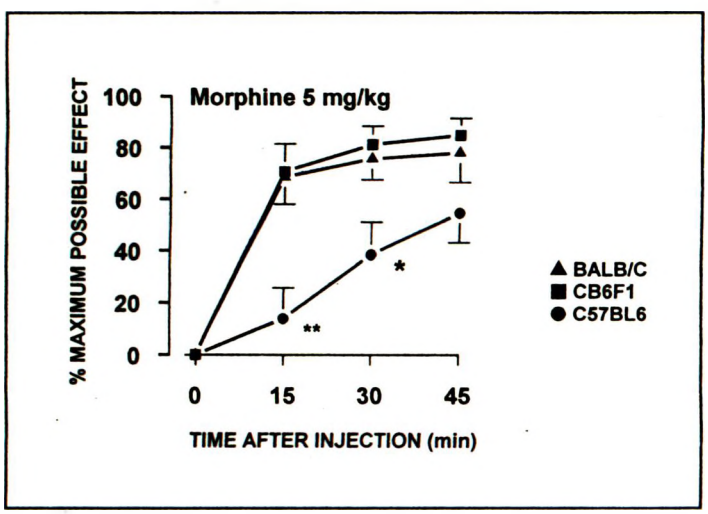

Fig. 1:
The antinociceptive effects of morphine (5 mg/kg) in mice of BALB/C, C57BL/6 and CB6F1 strains.
** significantly different than that of BALB/C mice (P<0.05).*
*** significantly different than that of BALB/C mice (P<0.001).*

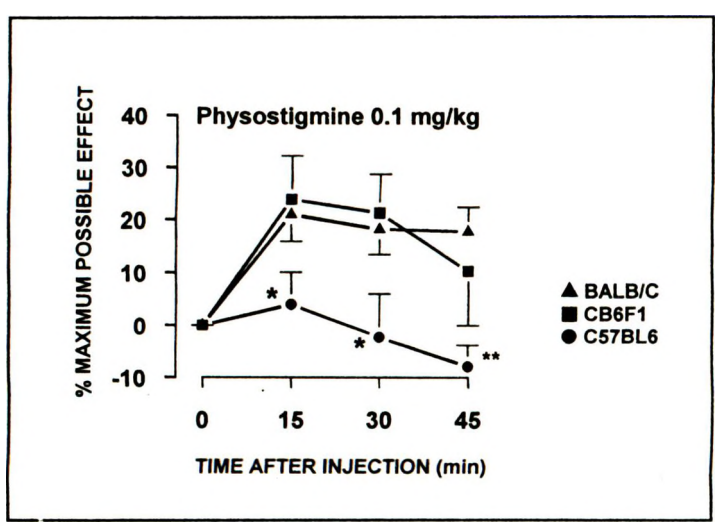

Fig. 2:
The antinociceptive effects of physostigmine (0.1 mg/kg) in mice of BALB/C, C57BL/6 and CB6F1 strains.
** significantly different than that of BALB/C mice (P<0.05).*
*** significantly different than that of BALB/C mice (P<0.001).*

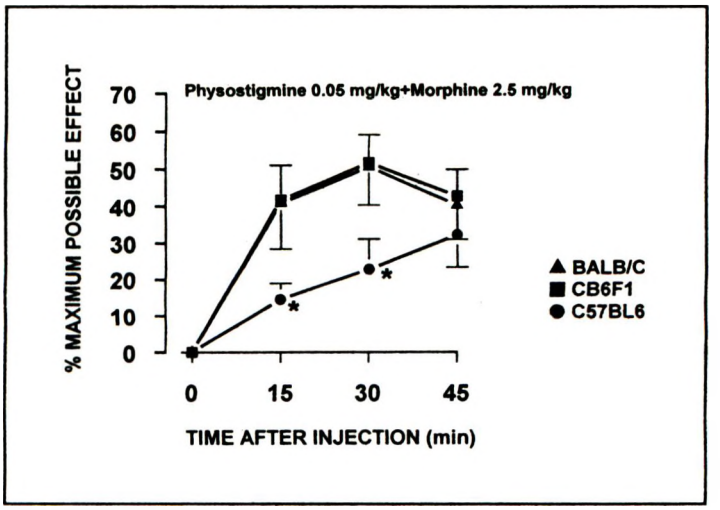

Fig. 3:
The antinociceptive effects of morphine (2.5 mg/kg) and physostigmine (0.05 mg/kg) in combination in mice of BALB/C, C57BL/6 and CB6F1 strains.
** significantly different than that of BALB/C mice (P<0.05).*

DISCUSSION

The present study shows that there are significant strain differences in both opioid and cholinergic analgesia in mice. C57BL/6 mice were found to be significantly less responsive to physostigmine and morphine than albino BALB/C and CB6F1 strains, though the basal tail flick latencies did not show any significant difference among different strains. Our results are in agreement with Pavone et al. (9) who have reported that oxotremorine (0.005 mg/kg) was ineffective in C57BL/6 mice whereas it caused a significant increase in TFL in DBA/2 strain. 5-methoxy-N, N-dimethyltryptamine, a serotonergic receptor agonist was also found to have a lower analgesic effect in C57BL/6 mice (10). Although this mutant strain serves as an animal model of hypocholinergic hippocampal functioning (11), all of these observations indicate that C57BL/6 mice are hyporesponsive not only to cholinergic agonists but also to morphine and serotonin. Indeed, a strain dependent difference in serotonin - induced nociception was also reported in rats (10). Interestingly, cross mice (CB6F1) was as sensible to both morphine and physostigmine as albino BALB/C suggesting that this behaviour was inherited.

The existence of a muscarinic cholinergic synapse within the opioid pain inhibitory pathway has been suggested since scopolamine blocks opioid analgesia and naltrexone attenuates the antinociceptive effects of oxotremorine (7). Physostigmine has been reported to potentiate morphine - induced antinociception (5). A synergistic interaction between morphine and physostigmine was demonstrated in BALB/C and CB6F1 strains in this study, but C57BL/6 mice were found to be less sensitive to morphine - physostigmine combination as much as morphine alone. These results indicate that not only opioid antinociception, but also cholinergic influence on opioid analgesia are strain-dependent in mice.

In conclusion, the present results showing significantly less analgesic effect of morphine, physostigmine and morphine-physostigmine combination in C57BL/6 mice suggest a strain - dependent modulation of nociception. The hyporesponsiveness to those analgesics seems to be a recessively inherited behaviour and may be due to a different neural organization which regulates the transmission and / or perception of painful stimuli in CNS. These results also stress on the importance of use of inbred animals for nociception studies.

REFERENCES

1. *Ham RJ, Knisely JS. Development of differences in the analgesia produced by the central cholinergic system. Develop Psychobiol 1986;20:345-354.*
2. *Ireson JD. A comparison of the antinociceptive action of cholinomimetic and morphine - like drugs. Br J Pharmacol 1970;40:92-99.*
3. *Bartolini A, Bartolini R, Aiello - M P, Biscini A, Renzi G. New data concerning the interaction between cholinergic, enkephalinergic and serotonergic systems during analgesia. In: Fürst S, ed. Opiate Receptors and the Neurochemical Correlates of Pain. Akademiai Kiado, Budapest : Pergamon Press, 1980: 171 - 181.*
4. *Bartolini A, Galli A, Ghelardini C, Giotti A, Malcancio M, Malmberg-A P, Zucchi PL. Antinociception induced by systemic administration of local anaesthetics depends on a central cholinergic mechanism. Br J Pharmacol 1987;92:711-721.*
5. *Dirksen R, Nijhius GMH. The relevance of cholinergic transmission at the spinal level to*

opiate effectiveness. Eur J Pharmacol 1983;91:215-225.

6. *Gorth Jr T, Jansson I, Hartvig P, Gillberg PG, Post C. Interactions between noradrenergic and cholinergic mechanisms involved in spinal nociceptive processing. Acta Anaesthesiol Scand 1989;33:39-47.*
7. *Ladinsky H, Consolo S, Peri G, Crunelli V, Samanin, R. Pharmacological evidence for a serotonergic-cholinergic link in the striatum. In: Jenden, DJ, ed. Cholinergic mechanisms and psychopharmacology, Advances in Behavioral Biology, Vol. 24, New York: Plenum, 1977:615 - 622.*
8. *Ossipov MH, Gebhart GF. Opoid, cholinergic and alfa-adrenergic influences on the modulation of nociception from the lateral reticular nucleus of the rat. Brain Res 1986;384:282-293.*
9. *Pavone F. Consorti D, Fagioli S. Developmental differences of antinociceptive effects of oxotremorine in two inbred strains of mice. Dev Brain Res 1989;49:156-160.*
10. *Pavone F, Fagioli S. Serotonergic influence on cholinergic - induced analgesia: two inbred strains of mice. Brain Res 1992;577:347-350.*
11. *Wimer RE, Wimer CC, Chernow CR, Belvanz BA. The genetic organization of neuron number in the pyramidal cell layer of hippocampal regio superior in house mice. Brain Res 1980; 196:59-77.*