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Serotonergic influence on cholinergic-induced analgesia: differences in two inbred strains of mice

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C57BL/6 (C57) and DBA/2 (DBA) inbred mice showed different analgesic responses to cholinergic stimulation. The simultaneous administration of muscarinic and serotonergic agonists, oxotremorine and 5-methoxy-*NN*-dimethyltryptamine (5-MeODMT), lowered the antinociceptive effect of the cholinergic drug in DBA mice, while no effects were detectable in the C57 strain. These results suggest a strain-dependent behavioural effect of the interaction of cholinergic and serotonergic neuronal systems.

Several data show physiological and behavioral interactions between cholinergic and serotonergic systems[17,31]. Manipulations of serotonin transmission affect acetylcholine (ACh) content in the cerebral cortex and striatum[3,11]. Hypotermia, tremor and salivation induced by oxotremorine are potentiated by alaproclate, a selective 5-HT uptake inhibitor[9,22], and a functional interaction between the serotonergic and cholinergic systems in mediating learning and memory has also been suggested[2,24]. As far as the analgesic response is concerned, several studies have examined the role of 5-hydroxytryptamine in the regulation of nociceptive information[1,4,21], but little is known about the possible interactions between cholinergic and serotonergic systems on this response.

The antinociceptive properties of cholinergic agonists are well known[8,12,18,23,32]. This kind of analgesia appears to be mediated by central muscarinic sites, even if the mechanisms involved have not yet been clarified. In a previous experiment[23] we investigated the effects of the muscarinic receptor agonist, oxotremorine, on nociception in two inbred strains of mice C57BL/6 and DBA/2, which present different behavioral responses to cholinergic drugs. A higher analgesic reaction to oxotremorine administration was present in DBA/2 mice in comparison with C57BL/6 strain. It seemed interesting to investigate, in the same two inbred strains, the possible modifications induced by the serotonergic receptor agonist, 5-methoxy-*N,N*-dimethyltryptamine (5-MeODMT), on the analgesic effect of oxotremorine.

Naive male mice belonging to the C57BL/6 (C57) and DBA/2 (DBA) strains (Charles River, Como, Italy) were used. Groups of 8 mice for each strain were tested at 70–80 days of age. Baseline pain threshold and post-drug pain sensitivity were measured according to the tail-flick procedure. Only mice showing a baseline latency from 3 to 5 s were tested. The cut-off was 10 s. Immediately after baseline latency (BL) mice were intraperitoneally injected with oxotremorine (0.005 and 0.01 mg/kg) or 5-MeODMT (0.5 mg/kg), administered alone or in combination. Combinations of drugs were given as mixed solutions, in a single injection. A per se ineffective dose of 5-MeODMT was chosen to exclude its effects on other behavioral parameters, which could hide and/or interfere with the analgesic response. As control groups saline injected mice were tested. Tail-flick latencies (TFL) were measured 15, 30 and 60 min after drug administration and expressed as a percentage of maximum possible effect, $\%MPE = (TFL - BL)/(10 - BL) \times 100$. The data were analyzed by a two-way ANOVA for repeated measures, factors being Drug (six levels) and Time (three levels). Post-hoc comparisons were carried out with the Duncan multiple range test.

The administration of oxotremorine induced a dose- and strain-dependent analgesic effect and the simultaneous administration of an ineffective dose of the serotonergic agonist reduced the response in DBA strain (Fig. 1).

In particular the analysis of variance (ANOVA two-way) performed on the tail-flick latencies of C57 mice revealed a significant drug effect ($P < 0.01$) and a significant Drug × Time interaction ($P < 0.05$). Duncan post-hoc analysis showed a significant effect ($P < 0.01$)

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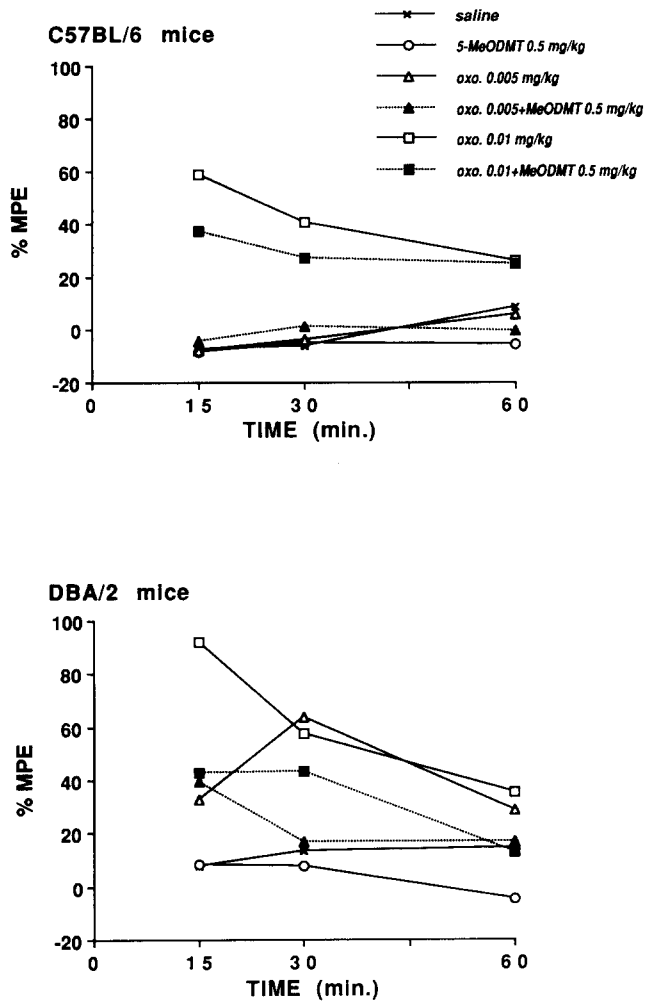

Fig. 1. Pain sensitivity in mice of the C57BL/6 and DBA/2 strains, receiving 5-methoxy-*N,N*-dimethyltryptamine (5-MeODMT: 0.5 mg/kg), oxotremorine (oxo: 0.005 and 0.01 mg/kg) alone or in combination. Post-drug tail-flick latencies, expressed as a percentage of maximum possible effect (% MPE), were measured 15, 30 and 60 min after injection. Values are the means of 8 mice.

of 0.01 mg/kg of oxotremorine until 30 min after injection; when 5-MeODMT was added, the tail-flick latencies lowered, even if a difference from the control group persisted. The administration of 0.5 mg/kg 5-MeODMT, 0.005 mg/kg oxotremorine and their combination, did not induce modifications in pain threshold.

When the effects in DBA mice are considered, two-way ANOVA showed significant Drug ($P < 0.01$), Time ($P < 0.01$) and Drug × Time interaction ($P < 0.05$). Duncan post hoc analysis revealed a significant effect for both doses of oxotremorine after 15 and 30 min. The serotonergic agonist, which is ineffective when administered alone, partially antagonized oxotremorine-induced analgesia: in fact the enhancement of tail-flick latencies after both doses of the muscarinic cholinergic agonist was abolished at 30 min testing time.

The results confirm a different nociceptive response to cholinergic stimulation by C57 and DBA mice and suggest, for the latter strain, an interaction of cholinergic and serotonergic system in modulating pain perception. No effect of the serotonergic agonist 5-MeODMT on oxotremorine-induced analgesia was observed in C57 mice.

Interactions between cholinergic and serotonergic systems have widely been described[5,17]. The 5-HT receptor agonists quipazine and *d*-fenfluramine increased Ach levels in the striatum and hippocampus in vivo[25], and depletion of 5-HT potentiated Ach release in vivo[30]. As far as influences on nociception are concerned, the cholinergic and serotonergic systems are both involved in pain modulation and are both able to influence drug- and environmentally-induced analgesia[14,15,21,27,32]. In particular both cholinergic and serotonergic drugs interfere with opioid-induced analgesia. 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[17]. On the other hand the 5-HT stimulants, quipazine and fluoxetine, enhance the

antinociceptive effects of morphine[19], while the antinociceptive properties of morphine are antagonized by the serotonergic antagonist, metergoline[10]. Moreover the opioid footshock-induced analgesia can be attenuated by depletion of brain 5-HT by parachlorophenylalanine[29]. Thus it can be gathered that the potentiation of serotonergic neurotransmission is accompained by a decrease in the sensitivity and reactivity to pain and by an improvement of the action of analgesics. However, more recently, both inhibition and facilitation of the spinal nociceptive reflexes have been reported after serotonergic manipulation, depending on the receptor subtype involved[28,33]. On the basis of anatomical and electrophysiological studies showing that some nuclei of the central nervous system share serotonergic and cholinergic receptors[5,6], we can hypothesize that these neuronal systems probably interact within these brain nuclei. One of these, the nucleus raphe magnus, is known to have heterogeneous populations of neurons, whose bulbospinal projections are involved in nociceptive modulation[13,16]. In fact, in addition to the serotonergic raphe-spinal descending fibres, also several cholinergic descending pathways belong to the raphe[5,8]. Iontophoretically applied Ach activated neurons in the nucleus raphe magnus and microinjection of the cholinergic agonist carbachol produced antinociception[6]. The serotonergic agonist 5-MeODMT could interfere with one of the cholinergic descending pathways, stimulated by oxotremorine, inhibiting its action on nociception. Our results seem to be in line with data showing a decrease in Ach release due to the serotonergic agonist which could counteract the antinociceptive action of oxotremorine. This would be in agreement also with data showing a potentiation of other effects of oxotremorine by alaproclate[22].

As far as the differences observed between the two inbred strains are concerned, it has been noted that, as previously demonstrated, they react differently to oxotremorine stimulation, with the DBA strain more sensible to its effects, and that these differences have been correlated with strain-dependent structural and functional organization of cholinergic system[7,20,23,26]. We can hypothesize that the lack of interference between the serotonergic and the cholinergic agonists observed in C57 mice is a consequence of a different sensitivity of this strain also to serotonergic stimulation or of a different neural organization which regulates the transmission and/or perception of painful stimuli in CNS.

In summary, the present results showing a reduction of oxotremorine-induced analgesia by a serotonergic agonist only in one of the inbred strains used suggest a strain-dependent modulation of nociception by the interaction of serotonergic and cholinergic systems.

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