Action of Opioid Agonist-antagonist Drugs on the Pupil and Nociceptive Responses in Mice

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Opioid derivatives with mixed agonist-antagonist activities are becoming increasingly more popular in analgesia. We tested the mydriatic and analgesic activity of morphine in mice in comparison with similar effects of three agonist-antagonist agents: buprenorphine, butorphanol and nalbuphine. We also examined the antagonistic action of these three drugs by evaluating the analgesia and mydriasis in animals pretreated with morphine.

The analgesic effect was assayed using the hot plate method while the pupillary responses were measured with a binocular operating microscope.

Morphine produced dose-dependent mydriasis and analgesia in mice. The morphine-type agent buprenorphine and two nalorphine-type agonist-antagonists, butorphanol and nalbuphine, caused agonistic mydriatic and analgesic effects, usually less effective then morphine. Buprenorphine proved to have higher agonist activity than butorphanol and nalbuphine. The difference between butorphanol and nalbuphine was not statistically significant.

A correlation between the mydriatic and the analgesic activity, known to exist among opioid derivatives with agonist activity only, was also demonstrated in the three investigated agonist-antagonist agents.

Morphine-induced mydriasis and analgesia were reversed by all three agonist-antagonist drugs, but buprenorphine is a significantly weak antagonist in comparison with butorphanol and nalbuphine. An antagonistic property (antimydriatic and antianalgesic effects after pretreatment with morphine) of both nalorphine-type investigated drugs was not statistically significant, except for the antianalgesic effect of nalbuphine in doses 1 and 3 $mg \cdot kg^{-1}$, which was higher in comparison with butorphanol. (Key words: morphine, buprenorphine, butorphanol, nalbuphine, agonist-antagonist opioids, pupil, analgesia, mydriasis)

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Experiments on mice showed that specific effects of opioids include analgesia and pupillary mydriasis[1,2]. Janssen and Jagenau[1] showed in a large series of opioid derivatives an impressive correlation between analgesia and mydriasis. In recent years, the new group of synthetic agonist-antagonist drugs attracted due attention, and these agents became increasingly popular analgesic agents. It is interesting to examine whether these

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agents have mydriatic effects too. In addition, we have previously shown the suitability of the pupillary responses as a measure of the antagonist activity of several opioid antagonists[3]. Since the new agents have intrinsic antagonistic actions, they could interfere with morphine-induced mydriasis and analgesia. The present paper reports on the results of a series of experiments designed to test these actions.

Materials and Methods

1. *Evaluation of mydriatic and analgesic effects*

Male albino ICR mice weighing 20–25 grams were used in all experiments. Morphine and three evaluated agonist-antagonist drugs were injected intraperitonealy in volumes of 0.2 ml containing doses of 1, 3, 10 and 30 $mg \cdot kg^{-1}$ in dissolved saline. Nine animals in the control group were injected with equal volumes (0.2 ml) of saline. Each experiment in each dose of the examined agents was performed on nine animals. Measurement of the pupillary diameter using a binocular operating microscope, employed methods similar to those described earlier[2]. The analgesic effect was assayed using the hot plate method[4]. Mydriatic and analgesic effects of each of the agonist-antagonist was compared with those of morphine in similar doses, and with each of the other two agonist-antagonist agents.

The INDEX OF ANALGESIA (IA) was calculated from the formula

$$IA = \frac{t - t_o}{30 - t_o}$$

where t and t_o are the reaction times of the same animal when medicated and when not treated, respectively. Animals with t_o exceeding 15 seconds were discarded. If after the introduction of the test drug no reaction followed during 30 seconds exposure on the hot plate, the experiment was terminated and 30 seconds was taken as the reaction time, giving IA=1.

For investigation of correlation between mydriatic and analgesic activity of the four test drugs the median effective dose (ED_{50}) for mydriatic and analgesic effects was calculated after construction of quantal logarithmic dose-effect curves[5].

2. *Evaluation of antimydriatic and antianalgesic effects*

A. Antimydriatic effect

Morphine 10 $mg \cdot kg^{-1}$ intraperitoneally was injected and 15 minutes thereafter pupil diameter was measured. One of the investigated drugs was injected intraperitoneally immediately after registering the mydriatic effect of morphine. 15 min after injection of the antagonist, the pupillary diameter was measured repeatedly. Antimydriatic effect of each agonist-antagonist was evaluated in doses of 1, 3, 10 and 30 $mg \cdot kg^{-1}$. 10 mg of injected morphine and each evaluated dose of antagonist was dissolved in 0.1 ml of saline, so that each mouse received 0.2 ml intraperitoneally. Nine animals were taken for each experiment of each dose of antagonist and for the control group. In the control group normal saline 0.1 ml was injected in place of the antagonist.

The antimydriatic effect (AME) of the test drugs was defined as PER CENT OF ANTAGONISM, calculated from the following formula

$$\text{AME (PER CENT OF ANTAGONISM)} = \frac{y - z}{y} * 100$$

where y =pupil diameter 15 min after injection of morphine 10 $mg \cdot kg^{-1}$;
z =pupil diameter 15 min after antagonist injection and 30 min after 10 $mg \cdot kg^{-1}$ morphine pretreatment;

B. Antianalgesic effect

Antianalgesic effect was evaluated similarly to the antimydriatic, but the reaction time was measured in place of

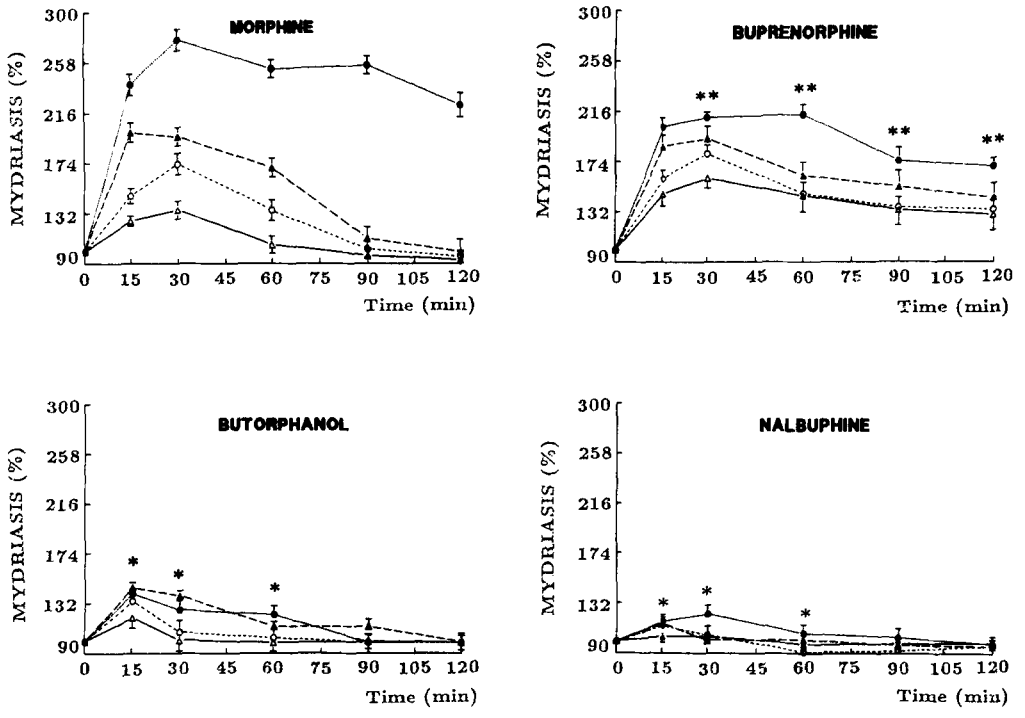

Fig. 1. Mydriatic Activity of Morphine and Three Investigated Drugs.

The diameter of the pupil before injection was taken as 100%. Each point in this and subsequent figures was derived from experiments on 9 animals, and vertical bars indicate standard errors of the mean.

Time (min) – the time after intraperitoneally injection of the agent.

△–△: 1 mg·kg^{-1}; ○–○: 3 mg·mg^{-1}; ▲–▲: 10 mg·kg^{-1}; ●–●: 30 mg·kg^{-1}

*significant statistical differences in comparison with similar doses of both morphine and buprenorphine.

**significant difference between morphine and buprenorphine in the dose of 30 mg·kg^{-1}.

diameter of the pupil.

The antianalgesic effect (AAE), calculated as the ANTAGONISTIC INDEX, was derived from the formula

$$\text{AAE (ANTAGONISTIC INDEX)} = \frac{t_{15mo} - t_x}{t_{15mo}} * 100$$

where t_{15mo} =reaction time 15 min after injection of 10 mg·kg^{-1} morphine; t_x =reaction time 15 min after antagonist injection and 30 min after 10 mg·kg^{-1} morphine pretreatment.

Statistical analysis was performed using t test for evaluation of the mean.

Results

1. *Mydriatic and analgesic activities*

Physiological saline has no significant effect on pupil size or nociceptive responses. Morphine and buprenorphine induced dose-dependent mydriasis (fig. 1) and analgesia (fig. 2). Butorphanol and nalbuphine had dose-dependent analgesic effects, but their mydriatic activity was less clearly dependent on the dose (figs. 1, 2). We examined the analgesic effect during 60 min only, because of the significant decrease of the analgesia, produced by

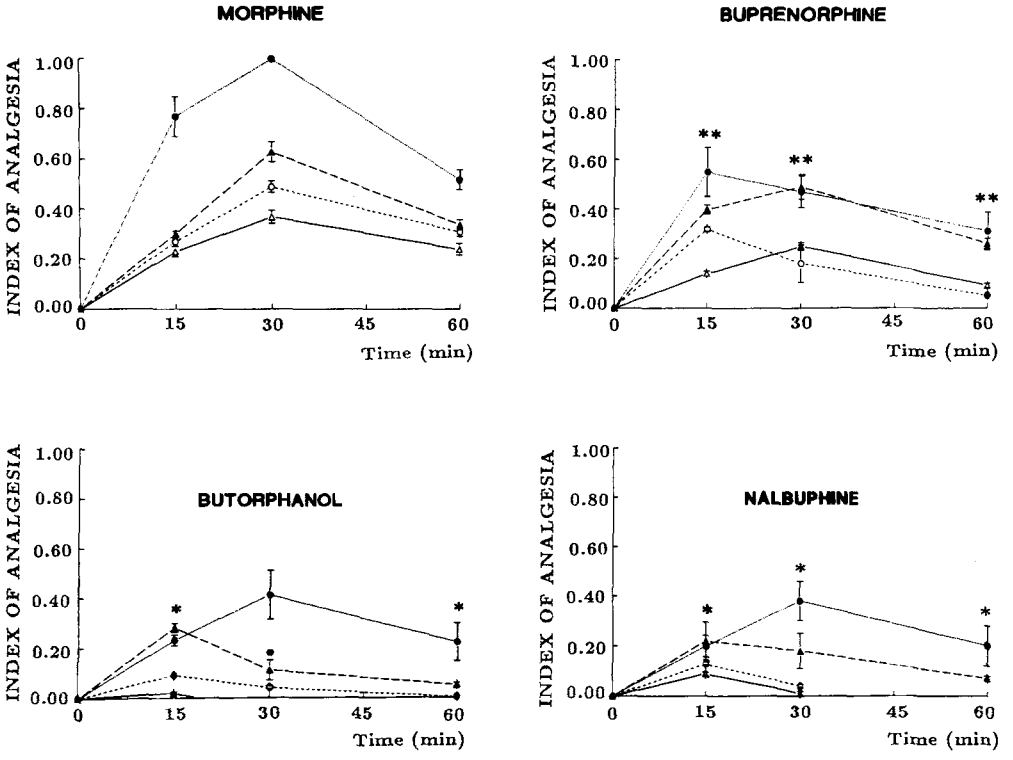

Fig. 2. Analgesic Activity of Morphine and Three Investigated Agents.
Time (min) - the time after intraperitoneally injection of the agent.
△–△: 1 mg·kg^{-1}; ○–○: 3 mg·mg^{-1}; ▲–▲: 10 mg·kg^{-1}; ●–●: 30 mg·kg^{-1}
*significant statistical differences in comparison with similar doses of both morphine and buprenorphine.
**significant difference between morphine and buprenorphine in the dose of 30 mg·kg^{-1}.

butorphanol and nalbuphine 60 min after injection. The mydriatic and analgesic effects peaked at 15–30 min (figs. 1, 2).

Mydriatic and analgesic activities of morphine and buprenorphine were significantly greater than those of butorphanol and nalbuphine. There were no significant differences between mydriatic as well as analgesic activities of morphine and those of buprenorphine in a dose of 1–10 mg·kg^{-1}. At the highest dose (30 mg·kg^{-1}) examined mydriatic and analgesic activities of morphine were significantly greater than those of buprenorphine. The difference between butorphanol and nalbuphine was not statistically significant (figs. 1, 2).

The correlation between the mydriatic and the analgesic activities was clear with respect to morphine and all three examined drugs (fig. 3).

2. *Antimydriatic and antianalgesic activities*

Morphine-induced mydriasis and analgesia could be reversed by the three test drugs (fig. 4 A, B). The antagonistic effect of buprenorphine was significantly less ($P < 0.05$) when compared with the other two test drugs, except for the antimydriatic effect of buprenorphine in the dose of 10 mg·kg^{-1}, and its antianalgesic effect in the dose of 30 mg·kg^{-1} (no significant difference with the same dose of butorphanol).

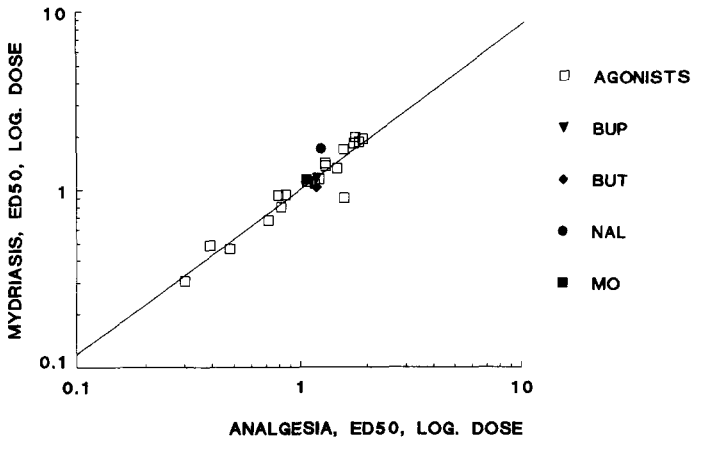

Fig. 3. Correlation between Analgesic and Mydriatic Activity of Opioid Agonists and Mixed Agonists-antagonists.

ED_{50} – median effective mydriatic and analgesic logarithm was investigated for each drug (morphine and three agonist-antagonists).

Results of ED_{50} (mydriasis and analgesia) of 20 opioids agonists, market in empty squares, were taked from the work of Janssen and Jagenau[1].

The difference between butorphanol and nalbuphine was not statistically significant, except for a weaker antianalgesic effect of butorphanol in the dose of 1 and 3 $mg \cdot kg^{-1}$ (fig. 4 A, B).

Discussion

Analgesia is a clinically superior property of both groups of drugs: pure opioid agonists and opioid agonist-antagonists. Morphine is a classical standard against which new analgesics are measured[6].

Morphine and other pure agonists cause miosis in man and dog due to an excitatory action on the nucleus of the oculomotor nerve[6,7]. In mice, cats and monkeys, morphine produces mydriasis[1–3,6]. This property of opioids is convenient for comparing new opioid derivatives with morphine, and opioids antagonists may be compared between them itself by its antimydriatic action in animals previously treated with morphine. In their work Janssen and Jagenau[1] conclude that there is a considerable correlation between the mydriatic and the analgesic activities of opioids in mice, but they have used only full opioid agonists.

Three agents of the group of opioid agonist-antagonists were investigated in our work. One of these agents, buprenorphine, belongs to the morphine type, and the two others to the group of nalorphine type[8–12].

It is not surprising that morphine (action on the mu opioid receptors)[12] and buprenorphine (partial agonist of mu receptors)[12] caused dose-dependent mydriasis and analgesia in mice (figs. 1, 2). Butorphanol and nalbuphine have a dose dependent analgesic action because they bring about an agonistic action on the kappa receptors, since the occupation of the kappa receptors by opioid derivatives may contribute to analgesia[6,12]. Note that all the three examined agents fit this general pattern, regardless of their inherent antagonistic activity.

All three investigated drugs caused mydriasis and analgesia in mice, similar to, but less effective, than morphine. The morphine type agonist-antagonist opioid buprenorphine had the highest agonist and the weakest antagonist activity in comparison with the other two agents, which belong to the nalorphine type agonist-antagonists. In Jasinski's work[12] a certain difference between opioid agonist-antagonists is argued in the relationship to the agents of the opioid receptors. However we have not found any statistically significant difference of the mydriatic and analgesic (agonistic) and/or antimydriatic and antianal-

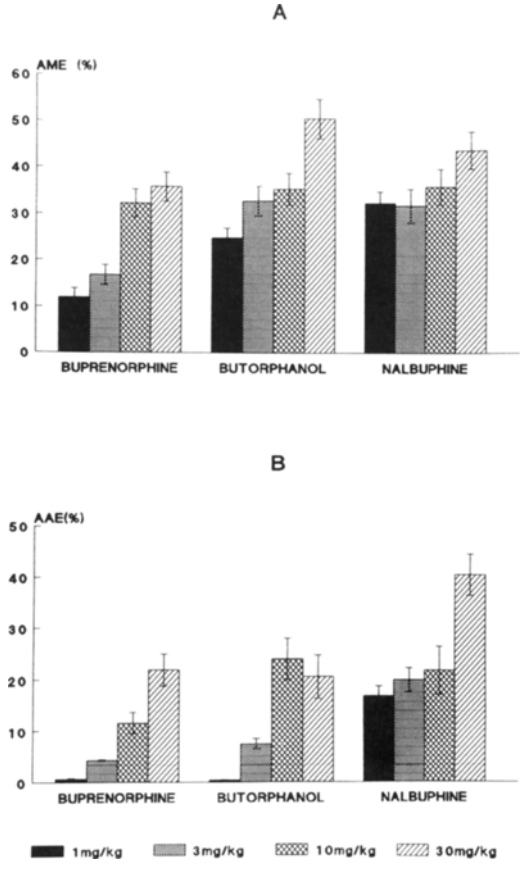

Fig. 4. A – Antagonistic Antimydriatic Effect of Three Investigated Agents.

AME (%) – antimydriatic effect, expressed as per cent of antagonism. Mydriasis was induced by morphine 10 $mg \cdot kg^{-1}$ and reversed by the one of the test drugs in doses of 1, 3, 10, and 30 $mg \cdot kg^{-1}$. Note the dose-related antagonism.

B – Antagonistic Antianalgesic Effect of Three Investigated Agents.

Analgesia was induced by 10 $mg \cdot kg^{-1}$ morphine, and 15 min later the investigated drugs were injected. The ANTAGONISTIC INDEX OR ANTAGONISTIC ANTIANALGESIC EFFECT [AAE (%)] was measured after an additional period of 15 min. Note that there is a clear dose response relationship for buprenorphine and nalbuphine, while butorphanol reached a peak after which subsequent dose increment resulted in a smaller amplitude of response, but this difference was not statistically significant.

gesic (antagonistic) effect between butorphanol and nalbuphine in mice.

The antagonistic effects of butorphanol and nalbuphine are dose dependent in the investigated doses (fig. 4).

We found that in three evaluated agonist-antagonist agents a strong correlation is present between mydriatic and analgesic properties, and this property that Jansen and Jagenau[1] found for full opioid agonists may be extended to agonist-antagonists opioids derivatives too.

Conclusions

1. All three investigated agonist-antagonists agents produce analgesic and mydriatic effect in mice. These effects are usually dose-dependent and weaker than with morphine.
2. The morphine type agent buprenorphine produces a higher analgesic and mydriatic effect in comparison with the nalorphine type drugs butorphanol and nalbuphine. Difference between butorfanol and nalbuphine was not found in this manner.
3. A strong correlation between the mydriatic and the analgesic activities was clearly demonstrated with regard to all three agonist-antagonists. This correlation was examined earlier as to pure opioids agonists only.
4. All three agonist-antagonist agents produced dose dependent antagonistic effects in animals that were pretreated with morphine.
5. The antagonistic property of buprenorphine was weaker in comparison with butorphanol and nalbuphine.
6. In doses of 1 and 3 $mg \cdot kg^{-1}$ nalbuphine produced a higher antianalgesic effect in compari-

son with similar doses of butorphanol.

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