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Tolerance to the analgesic effect of buprenorphine, butorphanol, nalbuphine, and cyclorphan, and cross-tolerance to morphine

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Abstract
Purpose. The increased use of opioids in the chronic treatment of pain, especially with oncologic patients, encourages the search for drugs with potent analgesic activity, but with minimal induced tolerance and cross-tolerance to morphine.
Methods. Four agonist-antagonist opioid derivatives (buprenorphine, butorphanol, nalbuphine, and cyclorphan) were examined. Tolerance to the analgesic effect of the four drugs and their cross-tolerance effects with morphine were evaluated in ICR albino mice by the "hot plate method". Measurements of the analgesic effect were taken before and after chronic treatment (of 14 days duration) with these drugs, as well as morphine.
Results. All tested drugs produced tolerance after 14 days of treatment. Chronic treatment with morphine reduced the effects of nalbuphine and cyclorphan, but not those of buprenorphine and butorphanol. After 14 days treatment with buprenorphine and cyclorphan, the analgesic action of morphine was reduced, but this reduction did not occur after butorphanol and nalbuphine treatments.
Conclusion. Of the four agonist-antagonists tested, butorphanol seems to be least liikely to produce cross-tolerance with morphine.

Key words Morphine · Buprenorphine · Butorphanol · Nalbuphine · Cyclorphan · Analgesia · Tolerance · Cross-tolerance · Opioids · Agonist-antagonist

Introduction

The use of opioids in chronic pain treatment demands a gradual increase in the dose, due to the development of tolerance [1–3]. It has always been desirable to identify an agent that has potent analgesic activity, but that does not induce tolerance. Newer opioid derivatives with partial agonistic activity or with agonist-antagonist activities are thought to possess this characteristic. Opioid derivatives are known to produce pupillary mydriasis in mice [4–8], and a correlation exists between the mydriatic and the analgesic effects of these agents [4,6]. Previously, we have demonstrated the development of tolerance to the mydriatic effects of buprenorphine, nalbuphine, and cyclorphan, as well as cross-tolerance to morphine. However, we did not observe tolerance to butorphanol or its cross-tolerance to morphine [8]. In the present work, we attempted to study whether chronic injections of these agents produced tolerance, and cross-tolerance to the analgetic effect of morphine.

Materials and methods

Male albino ICR mice, weighing 30–35 g, were used in all experiments. According to the previously used hot-plate method [6], each mouse was placed in a glass chamber, the floor of which was surrounded by warm water (55°C). Nociceptive reaction was defined by foot licking or an attempt to escape from the chamber. The time between the placement of each mouse into the chamber and the first sign of pain sensation was measured before and after drug administration. Five agents were investigated: buprenorphine, butorphanol, nalbuphine, cyclorphan, and morphine. Injection of saline was used as the control. Each agent was administrated chronically, i.e., for 14 days, to a group of ten mice, and the results averaged.

Mice were injected subcutaneously with equipotent doses of the opioid drugs (10 mg/kg of nalbuphine, butorphanol, and cyclorphane and 0.4 mg/kg of buprenorphine in 0.2 ml solution). These doses were selected from the information given in our previous works [6,8]. Nociceptive reactions were measured 15, 30, 60, 90, and 120 min after injection. Responses were calculated as

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percentages of the baseline data values (day 1) (i.e., mean latency prior to drug administration was taken to be 100%).

For the induction of tolerance, group A was injected with 1.2 mg/kg of buprenorphine or 30 mg/kg of the other three agents twice daily for 14 days. Group B was injected similarily with a high dose of morphine, 200 mg/kg twice daily, while group C—the control group—was injected with saline (0.2 ml) twice daily. Sixteen hours after the last injection, the tested drug was administered. Nociceptive reactions were measured at the same time intervals over a 2-h period. Four hours after the animals had recovered from the effect of the test drug, 10 mg/kg morphine was injected to all animals, with subseqent nociceptive testing. These tests provided information about the presence of cross-tolerance to morphine in animals after chronic administration of one of the evaluated agents.

Statistical analysis was performed using analysis of variance (ANOVA; single factor variances for evaluation of mean) and the *t*-test (two samples assuming equal variances), comparing the effects at each of the time points measured, as well as the areas under the pain-reaction curves.

Results

All four evaluated drugs produced distinctive analgesic effects in the three groups of mice, with a maximal action at 30 min, continuing for at least 120 min. After chronic treatment for 14 days with each of the evaluated agents (Tables 1A–4A), but not in the controls (Tables 1D–4D), tolerance to the analgesic effect had developed. Cross-tolerance to the analgesic effect of morphine was observed after chronic treatment with

Table 1. Analgesic effects of buprenorphine

A. Tolerance to antinociceptive action by chronic buprenorphine injection

	Time from injection until measurement of the analgesic effect					
	15 min	30 min	60 min	90 min	120 min	AUC
Nociceptive response to buprenorphine on day 1	171 ± 10	166 ± 6	142 ± 6	128 ± 6	116 ± 7	16892 ± 623
Nociceptive response to buprenorphine on day 16	126 ± 4*	121 ± 7*	112 ± 6*	126 ± 15	111 ± 7	14145 ± 623*

B. Cross-tolerance from chronic morphine injection

	Time from injection until measurement of the analgesic effect					
	15 min	30 min	60 min	90 min	120 min	AUC
Nociceptive response to buprenorphine on day 1	140 ± 6	147 ± 12	130 ± 10	113 ± 5	100 ± 4	14945 ± 744
Nociceptive response to buprenorphine on day 16	144 ± 20	120 ± 12	135 ± 12	126 ± 12	130.2 ± 13**	15386.1 ± 1247

C. Cross-tolerance to morphine by chronic buprenorphine injection

	Time from injection until measurement of the analgesic effect					
	15 min	30 min	60 min	90 min	120 min	AUC
Nociceptive response to morphine on day 1	159 ± 6	177 ± 11	136 ± 6	119 ± 3	105 ± 4	16325 ± 573
Nociceptive response to morphine on day 16	108 ± 5	110 ± 4	103 ± 5	99 ± 3	97 ± 4	12356 ± 278

D. Control

	Time from injection until measurement of the analgesic effect					
	15 min	30 min	60 min	90 min	120 min	AUC
Nociceptive response to buprenorphine on day 1	139 ± 4	157 ± 4	124 ± 7	110 ± 5	98 ± 2	14908 ± 322
Nociceptive response to buprenorphine on day 16	159 ± 6	160 ± 5	133 ± 5	116 ± 4	107 ± 4	15796 ± 389

* $P < 0.01$ vs day 1; ** $P < 0.05$ vs day 1
Data values are presented as percentages of the baseline nociceptive response (means ± SD)
AUC, Area under the curve

Table 2. Analgesic effects of nalbuphine

A. Tolerance to antinociceptive action by chronic nalbuphine injection

	Time from injection until measurement of the analgesic effect					
	15 min	30 min	60 min	90 min	120 min	AUC
Nociceptive response to nalbuphine on day 1	156 ± 7	202 ± 10	169 ± 21	126 ± 5	115 ± 5	18206.5 ± 891
Nociceptive response to nalbuphine on day 16	108 ± 10*	96 ± 8*	111 ± 8**	116 ± 9	99 ± 10	28195 ± 773

B. Cross-tolerance from chronic morphine injection

	Time from injection until measurement of the analgesic effect					
	15 min	30 min	60 min	90 min	120 min	AUC
Nociceptive response to nalbuphine on day 1	142 ± 12	172 ± 15	146 ± 14	110 ± 6	101 ± 6	15936 ± 590
Nociceptive response to nalbuphine on day 16	102 ± 14	99 ± 7*	98 ± 9*	104 ± 8	103 ± 7	12123 ± 640

C. Cross-tolerance to morphine by chronic nalbuphine injection

	Time from injection until measurement of the analgesic effect					
	15 min	30 min	60 min	90 min	120 min	AUC
Nociceptive response to morphine on day 1	142 ± 9	166 ± 14	136 ± 8	109 ± 9	117 ± 8	15662 ± 835
Nociceptive response to morphine on day 16	240 ± 48	189 ± 27	166 ± 25	162 ± 25	172 ± 40	21035 ± 3148

D. Control

	Time from injection until measurement of the analgesic effect					
	15 min	30 min	60 min	90 min	120 min	AUC
Nociceptive response to nalbuphine on day 1	195 ± 24	214 ± 15	186 ± 25	167 ± 18	102 ± 6	20599 ± 1306
Nociceptive response to nalbuphine on day 16	197 ± 20	225 ± 45	200 ± 24	146 ± 20	114 ± 14	20950 ± 2534

* $P < 0.01$ vs day 1; ** $P < 0.05$ vs day 1
Data values are presented as percentages of the baseline nociceptive response (means ± SD)
AUC, Area under the curve

nalbuphine and cyclorphan (Tables 2B; 4B), but not after the treatment with buprenorphine and butorphanol (Tables 1B; 3B).

We also evaluated the analgesic action of morphine after 14 days treatment with each of the evaluated agents (cross-tolerance). After treatment with buprenorphine and cyclorphan, the analgesic action of morphine was reduced (Tables 1C; 4C), but no cross-tolerance was seen after treatment with nalbuphine and butorphanol (Tables 2C; 3C). Conversely, chronic treatment with morphine resulted in tolerance to buprenorphine and cyclorphan, but not to the other two partial agonists (Table 5).

Discussion

Partial opiate agonists have become increasingly popular in clinical practice, because of their excellent analgesic effect and alleged reduced ability to induce dependence. Morphine and other pure agonists cause characteristic miosis in humans, rabbits, dogs, and most other animals. However, in mice, rats, and monkeys, morphine produces mydriasis [4–7]. Using only complete opioid agonists, Janssen and Jagenau [4] observed a remarkable correlation between the mydriatic and the analgesic effects of opioids in mice. Previously, we have characterized the pupillary actions of partial opioid agonists and the tolerance that develops after repeated administration. In the present study, we investigated, using a similar technique, the analgesic effects of four opioids with partial agonistic actions. Buprenorphine and cyclorphan are mixed agonist-antagonists with predominant mu-receptor activity. Nalbuphine and butorphanol are more active on kappa than on mu receptors [9–11,16–18].

As expected, chronic treatment with all four agents resulted in significant tolerance, most markedly

Table 3. Analgesic effects of butorphanol

A. Tolerance to antinociceptive action by chronic butorphanol injection

	Time from injection until measurement of the analgesic effect					
	15 min	30 min	60 min	90 min	120 min	AUC
Nociceptive response to butorphanol on day 1	160 ± 10	173 ± 9	166 ± 18	154 ± 16	120 ± 10	18426 ± 903
Nociceptive response to butorphanol on day 16	117 ± 6*	126 ± 8	122 ± 7**	129 ± 10	110 ± 2	14544 ± 588*

B. Cross-tolerance from chronic morphine injection

	Time from injection until measurement of the analgesic effect					
	15 min	30 min	60 min	90 min	120 min	AUC
Nociceptive response to butorphanol on day 1	164 ± 13	146 ± 7	137 ± 8	119 ± 8	114 ± 6	15890 ± 591
Nociceptive response to butorphanol on day 16	134 ± 10	130 ± 10	114 ± 9	102 ± 16	107 ± 8	13765 ± 1031

C. Cross-tolerance to morphine by chronic butorphanol injection

	Time from injection until measurement of the analgesic effect					
	15 min	30 min	60 min	90 min	120 min	AUC
Nociceptive response to morphine on day 1	142 ± 9	172 ± 11	136 ± 8	108 ± 8	117 ± 8	15856 ± 2115
Nociceptive response to morphine on day 16	140 ± 12	147 ± 11	125 ± 7	114 ± 11	103 ± 9	14884 ± 3021

D. Control

	Time from injection until measurement of the analgesic effect					
	15 min	30 min	60 min	90 min	120 min	AUC
Nociceptive response to butorphanol on day 1	139 ± 6	136 ± 8	128 ± 5	124 ± 5	117 ± 3	15217 ± 362
Nociceptive response to butorphanol on day 16	151 ± 6	163 ± 13	130 ± 5	103 ± 4*	97 ± 7**	15136 ± 474

* $P < 0.01$ vs day 1; ** $P < 0.05$ vs day 1
Data values are presented as percentages of the baseline nociceptive response (means ± SD)
AUC, Area under the curve

with nalbuphine, and least with buprenorphine. No loss of effect was observed when control mice were injected with saline. Animals that received chronic treatment with nalbuphine and cyclorphan exhibited cross-tolerance to morphine as well. However, cross-tolerance to morphine was not observed after the chronic administration of buprenorphine or butorphanol.

The mechanism underlying the process of tolerance is unclear. Our results support the view that pharmacokinetic factors, such as enhanced drug disposal, are unlikely contributors, because in the tolerant state, the maximal effect was reduced, rather than the duration of the effect being reduced (Tables 1–4). Also, the cross-tolerance to morphine argues against a metabolic mechanism, because the five drugs have different metabolic pathways. The pharmacodynamic mechanisms responsible for these effects, and in particular, the issue of non-consistent cross-tolerance, deserve further studies.

It is interesting that the tolerance demonstrated here was different from that seen with the same agonists in their effects on the pupil. For example, in our previous study, we did not find any tolerance to the mydriatic effect of butorphanol in mice [8], whereas the present study demonstrated the development of significant tolerance to the analgesic activity of this agent. These findings further strengthen our view that pharmacodynamic, rather than pharmacokinetic, factors are involved in the development of tolerance, and they also suggest that the mechanisms responsible for the effects of opiates on the pupil are substantially different from those involved in analgesia.

We did not observe cross-tolerance to morphine after chronic butorphanol (Table 5). In previous studies [12,13], tolerance to butorphanol seemed to be dose-dependent, with tolerance developing with higher doses.

The repeated administration of low doses of butorphanol twice daily for 4 days in rats did not induce

Table 4. Analgesic effects of cyclorphan

A. Tolerance to antinociceptive action by chronic cyclorphan injection

	Time from injection until measurement of the analgesic effect					
	15 min	30 min	60 min	90 min	120 min	AUC
Nociceptive response to cyclorphan on day 1	152 ± 7	175 ± 11	141 ± 16	115 ± 10	99 ± 4	16138 ± 873
Nociceptive response to cyclorphan on day 16	101 ± 7*	112 ± 6*	104 ± 7**	101 ± 3	94 ± 6	12754 ± 914*

B. Cross-tolerance from chronic morphine injection

	Time from injection until measurement of the analgesic effect					
	15 min	30 min	60 min	90 min	120 min	AUC
Nociceptive response to cyclorphan on day 1	173 ± 9	221 ± 13	164 ± 10	128 ± 9	109 ± 6	18708 ± 764
Nociceptive response to cyclorphan on day 16	104 ± 9*	108 ± 6	103 ± 7*	99 ± 7*	100 ± 4	12306 ± 279*

C. Cross-tolerance to morphine by chronic cyclorphan injection

	Time from injection until measurement of the analgesic effect					
	15 min	30 min	60 min	90 min	120 min	AUC
Nociceptive response to morphine on day 1	188 ± 24	209 ± 25	189 ± 21	154 ± 16	122 ± 11	21714 ± 2030
Nociceptive response to morphine on day 16	98 ± 8*	94 ± 8*	102 ± 6*	98 ± 7*	93 ± 6	11903 ± 620*

D. Control

	Time from injection until measurement of the analgesic effect					
	15 min	30 min	60 min	90 min	120 min	AUC
Nociceptive response to cyclorphan on day 1	153 ± 11	185 ± 13	138 ± 13	125 ± 9	102 ± 5	16618 ± 912
Nociceptive response to cyclorphan on day 16	176 ± 21	191 ± 18	176 ± 12	155 ± 11	138 ± 10	19725 ± 1120

* $P < 0.01$ vs day 1; ** $P < 0.05$ vs day 1
Data values are presented as percentages of the baseline nociceptive response (means ± SD)
AUC, Area under the curve

Table 5. Summary of results

	Index of remaining analgesic action (percentage)[a]		
Investigated drug	Tolerance to itself[a]	Cross-tolerance to morphine[b]	Inverse cross-tolerance morphine to investigated drug[b]
Buprenorphine	84 ± 5	104 ± 9	77 ± 3
Nalbuphine	72 ± 6	78 ± 6	125 ± 20
Butorphanol	81 ± 6	85 ± 28	90 ± 4
Cyclorphan	78 ± 5	67 ± 3	59 ± 6

Data values are given as means ± standard error
[a] The index was calculated according to the formula

Index = (area under curve on day 16 after investigated agent injection)/(area under curve after the first day injection) × 100%

[b] The area under the curve of the nociceptive reaction (in percentages) was calculated through 120 min after agent injection. The baseline value for time in seconds between the placement of each mouse into the chamber and the first sign of nociception before drug administration was taken to be 100%. Responses after 15, 30, 60, 90, and 120 min were calculated in percentages relative to the baseline data values

tolerance, in comparison with findings for nalbuphine, morphine, and buprenorphine, and no cross-tolerance between butorphanol and morphine was observed in these animals [15]. The findings of the present study demonstrate that longer treatment and higher doses of butorphanol lead to the development of tolerance and cross-tolerance.

Cyclorphan is a mixed agonist-antagonist with mu opiate receptor activity [16,17]. Tolerance to its analgesic activity was 78.3 ± 4.8% and cross-tolerance to morphine was 59.2 ± 6.3% (Table 5). Few studies deal with the receptor mechanisms responsible for cyclorphane effects. It is possible that multiple modes of action exist, which involve not only opiate receptors [16–18]. Cyclorphan and morphine have a similar affinity to mu receptors and to kappa receptors [8]. It is known that a high degree of cross-tolerance occurs in drugs with morphine-like action, but it seems that kappa agonists do not induce cross-tolerance to agents that act primarily on mu receptors [8].

In our previous study [8], we observed tolerance to the mydriatic effects of buprenorphine, nalbuphine, and cyclorphan, and cross-tolerance to morphine, but such tolerance and cross tolerance was not demonstrated with butorphanol. In the present study, tolerance to the analgesic effects of all the investigated agents was demonstrated, but cross-tolerance to morphine was absent after treatment with buprenorphine and butorphanol. It is known that delta opioid receptors are involved in the development of both tolerance and cross-tolerance [19]. We speculate that the lack of development of tolerance and cross-tolerance in buprenorphine and butorphanol is related to the different affinities of these agents to delta receptors [14,19].

In conclusion, tolerance and cross-tolerance to the analgesic effects of agonist-antagonist opioids commonly occur, with the notable exception of butorphanol, which does not seem to have cross-tolerance with morphine (i.e., the response to butorphanol is not affected by chronic morphine treatment, and the response to morphine is not affected by chronic butorphanol treatment).

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