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The General Anesthesia Induced by Various Drugs Differentially Affects Analgesia and Its Variability

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BANKS, W. A., T. L. TRENTMAN, A. J. KASTIN AND Z. H. GALINA. *The general anesthesia induced by various drugs differentially affects analgesia and its variability.* PHARMACOL BIOCHEM BEHAV 31(2) 397–403, 1988.— Responses to noxious stimuli in awake animals are not totally consensual but are influenced by environmental factors. We considered the possibility that the influence of the environment could be reduced by induction of general anesthesia. We, therefore, compared responses to nociceptive thermal stimuli by measuring tail flick latency, a spinal reflex, in anesthetized and awake mice. All anesthetics tested decreased the intraindividual variability in the measurement of response, suggesting that environmental factors may account for much of this variability in the awake mouse. Mice treated with pentobarbital showed a graded response to increasing levels of heat but were unresponsive to either morphine or naloxone. In mice anesthetized with pentobarbital, increases in latencies occurred only at very deep levels of anesthesia, while urethane increased and ketamine decreased latencies. The antinociceptive effect of urethane was unaffected by naloxone, but the nociceptive effect of ketamine was reversed by morphine. Thus, the various anesthetics could show differential effects towards opiate action. The decrease in statistical variability, the differential effects of general anesthetics on tail flick latency, and the distinctive effects of the different anesthetics on opiate action suggest that the anesthetized animal may be a useful tool in the study of nociception.

Analgesia Anesthesia Nociception Opiates Opiate receptors Naloxone Ketamine Pentobarbital Urethane Morphine

ANALGESIA, a condition in which nociceptive stimuli are no longer painful (12), may be achieved without loss of wakefulness. Similarly, some general anesthetics, such as barbiturates, are poor analgesics even when given in doses that result in unconsciousness (5). The response of an awake animal to noxious stimuli, however, is not totally consensual. An animal's environment influences its response to noxious stimuli and can lead to stress-induced (1) and stimulation-produced (14) analgesia. Such analgesia can complicate the study of the purely reflexive responses to nociception. We considered the possibility that unconscious animals would not be susceptible to many of the influences that complicate the study of analgesia in the awake animal. Therefore, we studied analgesia by measuring tail flick latency, a spinal reflex (8), in awake mice and in anesthetized mice.

METHOD

Male albino mice (CD-1) weighing about 20 g obtained from Charles River Labs (Wilmington, MA) were housed at least 48 hr in a room adjacent to the procedure room with a 12 hr light/12 hr dark lighting cycle and with food and water freely available. Before this, the mice had been housed in an animal facility in the same building under the same conditions. Mice were randomly assigned to the different categories of treatment. Tail flick latency was measured by placing the tail of a mouse over a 2 cm opening through which heat emanated from a nichrome thermal wire 0.7 mm in diameter with a length of 4.7 cm from the electrical pole to the opening. A manual switch activated a digital timer. Removal of the tail from the opening allowed the completion of a photoelectric circuit which deactivated the timer, with latency measured to the nearest 0.1 second. Exposure time was automatically stopped at 17 seconds since longer exposure can result in tissue damage to the tail. Awake mice were restrained in 2.5×5 cm Plexiglas tubes with one end sealed; anesthetized mice did not require restraint. Unless otherwise noted, tail flick latency was determined on each mouse at a voltage output through the wire of 2.08 volts (input of 121.6 volts AC with the rheostat setting at 80) after induction of

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TABLE 1

EFFECT OF MORPHINE AND NALOXONE ON TAIL FLICK LATENCIES IN AWAKE MICE AND MICE ANESTHETIZED WITH PENTOBARBITAL, URETHANE, OR KETAMINE

Injections		Time (min)				
First	Second	0	10	20	30	60
Saline	Saline	6.6 ± 1.2	8.0 ± 3.1	5.1 ± 0.9	9.1 ± 1.6	5.2 ± 0.9
Saline	Morphine	6.5 ± 0.7	9.2 ± 2.7	12.1 ± 2.9*	14.8 ± 2.2*	14.5 ± 1.0*
Saline	Naloxone	10.1 ± 0.7	9.1 ± 1.8	7.1 ± 3.3	6.4 ± 2.2	8.2 ± 3.0
Pento-barbital	Saline	6.2 ± 2.1	4.3 ± 0.9	6.2 ± 1.5	5.3 ± 1.3	5.6 ± 2.0
Pento-barbital	Morphine	5.5 ± 0.7	4.5 ± 0.6	4.0 ± 0.6	3.2 ± 0.4	4.2 ± 0.7
Pento-barbital	Naloxone	4.1 ± 0.8	4.3 ± 0.4	3.7 ± 0.5	5.1 ± 0.7	3.5 ± 0.3
Urethane	Saline	17 ± 0	17 ± 0	17 ± 0	17 ± 0	17 ± 0
Urethane	Naloxone	17 ± 0	17 ± 0	17 ± 0	17 ± 0	17 ± 0
Ketamine	Saline	4.3 ± 0.6	4.2 ± 0.7	3.8 ± 0.4	4.4 ± 2.9	3.9 ± 0.3
Ketamine	Morphine	3.3 ± 0.2	6.0 ± 2.8	6.0 ± 2.8	9.4 ± 3.1*	11.7 ± 2.4*

*$p<0.05$ compared with appropriate controls.

The values are the mean latencies ± the standard error. Mice received the first injection (anesthetic agent or saline) and latencies were determined (time 0 min) after the onset of general anesthesia (or 10 min after the injection of saline). Mice then received the second injection (saline, naloxone, or morphine) and latencies were determined 10, 20, 30, and 60 min later.

general anesthesia as determined by loss of spontaneous movements, rigid postures, and the righting reflex. When latencies were determined in triplicate for each mouse, the median value was used.

Effect of Various Anesthetics on Tail Flick Latency

Mice received saline (0.9%), urethane (2 g/kg), pentobarbital (65 mg/kg), or ketamine (200 mg/kg) IP in a volume of 10 ml/kg (n=11/group). At these doses, the anesthetics usually induced general anesthesia within 10 min, but the mice were reinjected as necessary to achieve anesthesia. Latency was measured after the onset of general anesthesia or 10–20 min after injection of saline.

Gradation of Response

Six mice were anesthetized with pentobarbital (65 mg/kg). Latencies were determined in triplicate for each mouse at the rheostat settings of 20 (an output of 0.43 volts), 40 (0.99 volts), 60 (1.64 volts), and 80 (2.08 volts).

Relationship Between Pre- and Postanesthetic Latencies

Mice were randomly assigned to one of three treatment groups of 24 mice each. Single latencies were determined and then each mouse received saline, ketamine, or pentobarbital. After onset of anesthesia or 10 min after treatment with saline, a single postinjection latency was determined. Latencies were determined singly rather than in triplicates in this study so that the paradigm would more closely resemble the one illustrated in Fig. 3.

Effects of Naloxone and Morphine on Latency

Mice (n=24) were randomly assigned to 6 groups. Three groups received saline and three groups received pentobarbital. Latencies were determined in triplicate after the onset of anesthesia or 10 min after administration of saline. This measurement was considered to be the latency at time 0 min (Table 1). Mice then received a second injection of saline, morphine (10 mg/kg), or naloxone (10 mg/kg) in a volume of 10 ml/kg. Latencies were determined 10, 20, 30, and 60 min after the second injection.

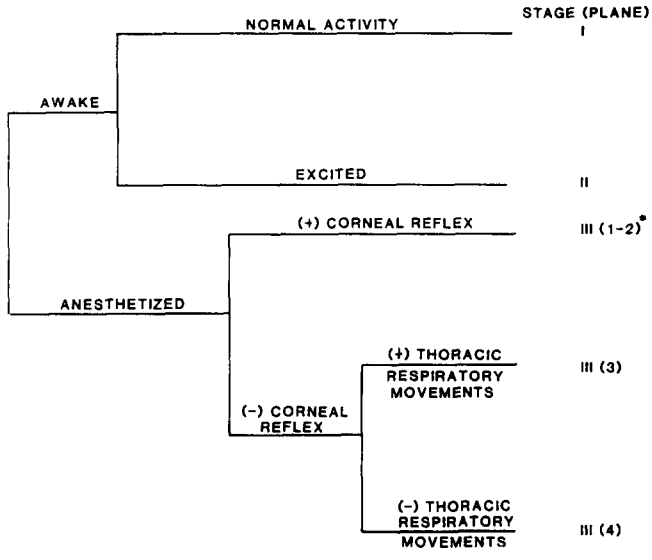

FIG. 1. Criteria used to determine stage and plane of anesthesia induced by pentobarbital. *Most commonly used for surgery.

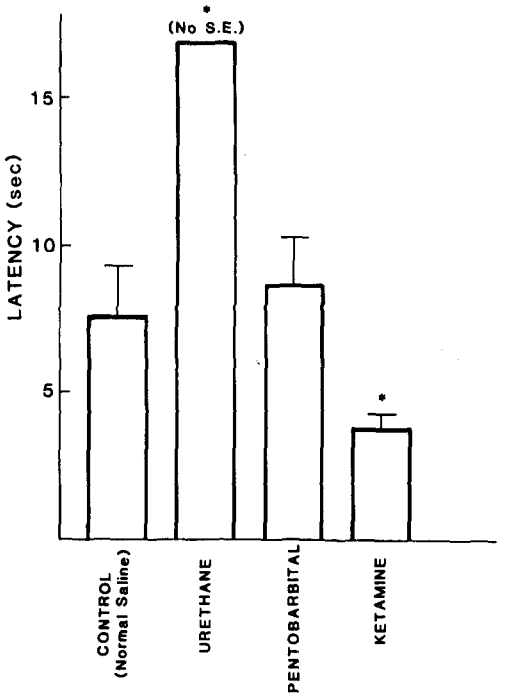

FIG. 2. Increased (urethane), decreased (ketamine), or unaltered (pentobarbital) levels of nociception as determined by tail flick latency in mice under general anesthesia in comparison with awake (control) mice that had received saline.

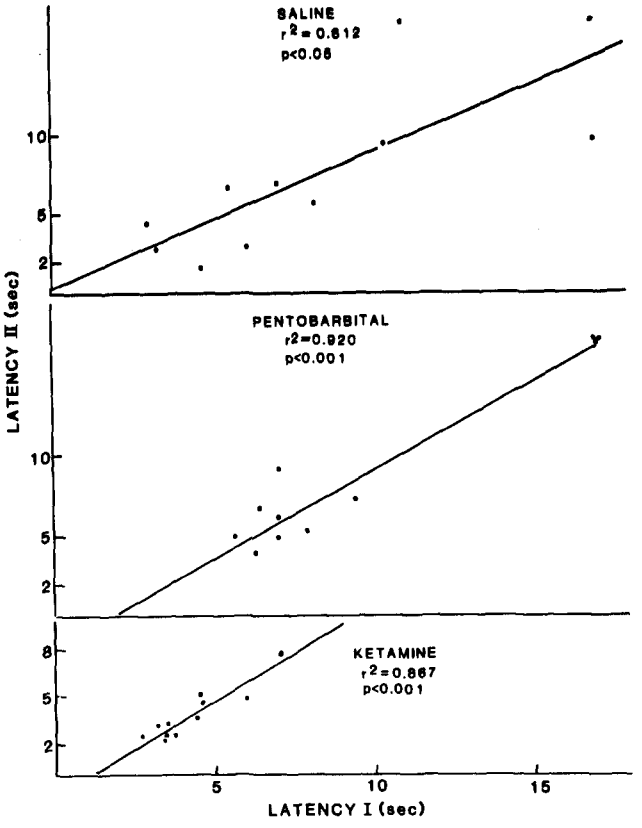

FIG. 3. The r^2 values for the correlation between first and second latencies was higher in mice anesthetized with pentobarbital or ketamine (r^2 of .920 and .867, respectively) than in mice that had received saline (r^2=.612), demonstrating less intraindividual variation in measurement.

Other mice anesthetized with urethane received either saline or naloxone (n=5/group). Morphine was not tested because mice anesthetized with urethane already exhibited maximal latencies (17 sec). Mice anesthetized with ketamine received either saline or morphine (n=5/group); naloxone was not tested because increased nociception was already being exhibited.

Effect of Varying Doses of Pentobarbital

Mice received one of the following doses of pentobarbital IP: 0, 8.1, 16.2, 32.5, 65.0, 97.5 mg/kg (n=7–8/group). Mice were classified by stage and plane 20 minutes after injection according to the criteria represented in Fig. 1. Pupillary reactions could not be determined in mice and so planes 1 and 2 of stage III were not differentiated. Latencies were determined in triplicate.

Latency During Induction of and Recovery From Pentobarbital Anesthesia

Ten mice were injected with 65 mg/kg of pentobarbital. Latency was determined before injection (preinjection latency), during the various stages of induction, and during recovery.

Statistical Analysis

Analysis of variance (ANOVA) was used to compare groups. If there were more than two groups, then Duncan's multiple range test (DMRT) was also used. Regression correlations were determined by the least squares method. The coefficient of variation is equal to the standard deviation multiplied by 100 and divided by the mean.

RESULTS

Effect of Various Anesthetics on Tail Flick Latency

ANOVA showed a statistically significant effect among the treatment groups, $F(3,41)=22.9$, $p<0.001$. DMRT showed that mice treated with urethane had significantly ($p<0.05$) longer latencies (17.0 ± 0.0) and mice treated with ketamine had shorter latencies (3.8 ± 0.04) than mice treated with saline (7.7 ± 1.6), while mice receiving pentobarbital were not statistically different (8.7 ± 1.6) from the group receiving saline (Fig. 2). This differs from other studies with ketamine, usually using subanesthetic doses, that have found an increase in latency (7, 13, 16). The coefficient of variation measuring interindividual variability in latencies among mice treated with saline (68.5%) was larger than the coefficients of variation among mice treated with ketamine (38.0%) or urethane (0%) but not pentobarbital (61.7%). This suggests that ketamine and urethane reduced interindividual variability in the measurement of latency.

Treatment with anesthetic agents also reduced the intraindividual variability for latency. The mean coefficients of variation of the triplicate determinations of latency was

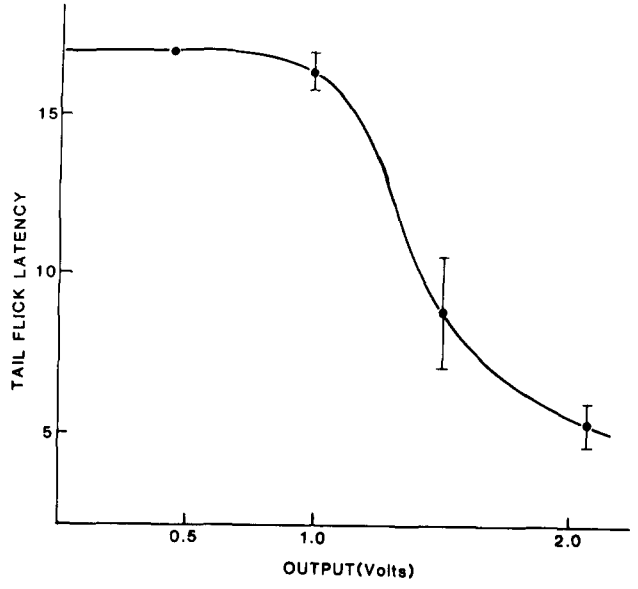

FIG. 4. Decreasing latency with increasing thermal stimulation as measured by the voltage output of the tail flick device in mice anesthetized with pentobarbital.

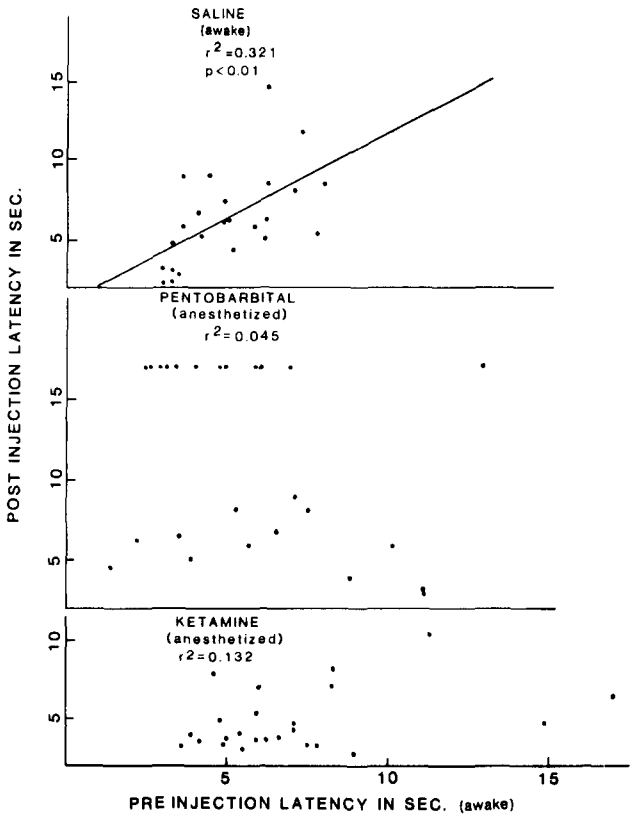

FIG. 5. Relationship between latencies preceding an IP injection of saline, pentobarbital, or ketamine and latencies taken 10–20 min later (after the onset of general anesthesia in the case of pentobarbital and ketamine). The statistically significant correlation in saline-treated (awake) mice but not in mice given general anesthesia suggests that anesthesia altered the factors that influenced nociception of the awake mice.

33±6.5% for mice treated with saline, 16.6±4.9% for mice treated with pentobarbital, 13.9±2.2% for mice treated with ketamine, and 0% for urethane since all latencies were 17.0. This decrease in variability for each mouse was also shown by the finding that the degree of correlation between the first and second, first and third, and second and third latencies of the triplicate determinations was higher for the anesthetized mice than for the unanesthetized mice. This is illustrated in Fig. 3, where the correlations between the first and second latencies, which are the determinations most analogous to the preinjection and postinjection schedule used in another experiment, are compared; the r^2 value was less for mice treated with saline (.612) than for mice treated with pentobarbital (.920) or ketamine (.867). Regression analysis could not be done for urethane since all values were 17.0.

Gradation of Response

Mice anesthetized with pentobarbital responded in a graded fashion to increasing heat (Fig. 4), $F(3,21)=32.4$, $p<0.001$. No mouse responded with the rheostat set at 20 (an output of 0.43 volts), while the mean response at a rheostat setting of 80 (2.08 volts) was 5.3±0.7.

Relationship Between Pre- and Postanesthetic Latencies

Figure 5 shows the relationships between tail flick latencies before and after injections. A correlation existed between the latencies before and after injection in mice that received saline. In mice that received either pentobarbital or ketamine, however, no relationship was found between the latency obtained before induction of general anesthesia and the latency obtained during general anesthesia.

Effects of Naloxone and Morphine on Latency

Morphine significantly increased tail flick latency in mice that had received saline but not in those anesthetized with pentobarbital (Table 1). The ANOVA was statistically significant for the one way effect of first injection (saline vs. pentobarbital), $F(1,3)=109$, $p<0.005$; the two-way interaction between first and second injections, $F(2,6)=8.7$, $p<0.05$; and the three-way interaction between first injection, second injection, and time, $F(8,24)=3.1$, $p<0.05$. DMRT showed that the 20 min, 30 min, and 60 min time points in saline/morphine-treated mice were significantly different from the 0 min time point in the saline/morphine-treated mice and from their respective 20 min, 30 min, and 60 min time points for latency in pentobarbital/morphine-treated mice. Treatment with naloxone did not produce any statistically significant effects in either saline pretreated or pentobarbital pretreated mice.

Naloxone was also without effect in mice anesthetized with urethane (Table 1). However, the increase in latency induced by morphine in mice anesthetized with ketamine reached statistical significance at 30 and 60 min (Table 1).

Effect of Varying Doses of Pentobarbital

Table 2 shows the relationship between dose of injected pentobarbital and level of anesthesia achieved at 20 min. Low doses produced no anesthesia, while high doses produced deep levels of anesthesia. Figure 6 shows the rela-

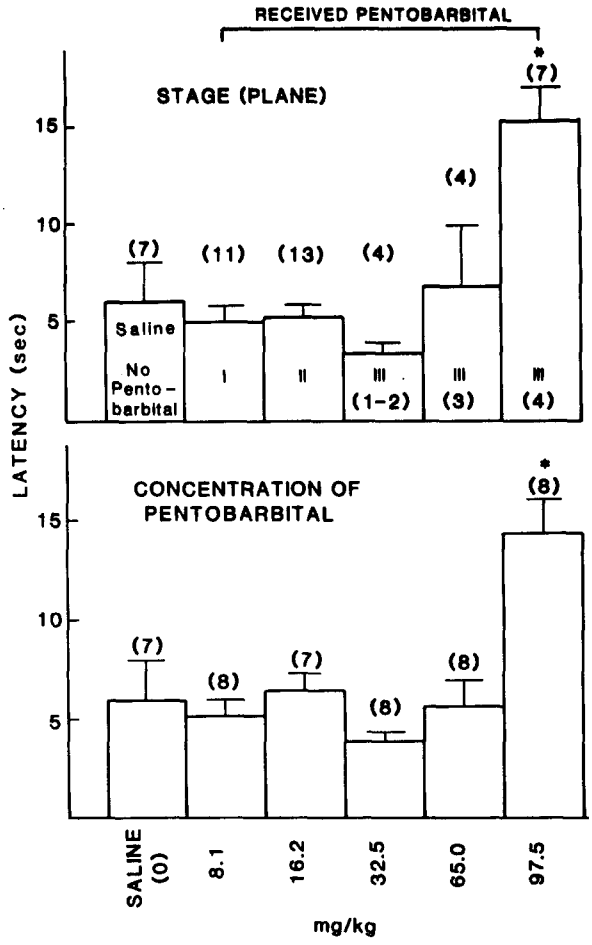

FIG. 6. Relationship between latency and level of anesthesia (upper panel) or dose of pentobarbital (lower panel). Only large doses of pentobarbital (97.5 mg/kg) or deep levels of anesthesia (stage III plane 4) resulted in significant increases in latency. The number in parentheses over a bar indicates the number of mice in the group and the number in parentheses inside the bar represents the plane.

TABLE 2

RELATIONSHIP BETWEEN STAGE (PLANE) OF ANESTHESIA AND DOSE OF PENTOBARBITAL ADMINISTERED IP

	Dose (mg/kg)				
Stage (plane)	97.5	65.0	32.5	16.2	8.1
I			2	1	8
II		1	6	7	
III (1–2)		4			
III (3)	1	3			
III (4)	7				

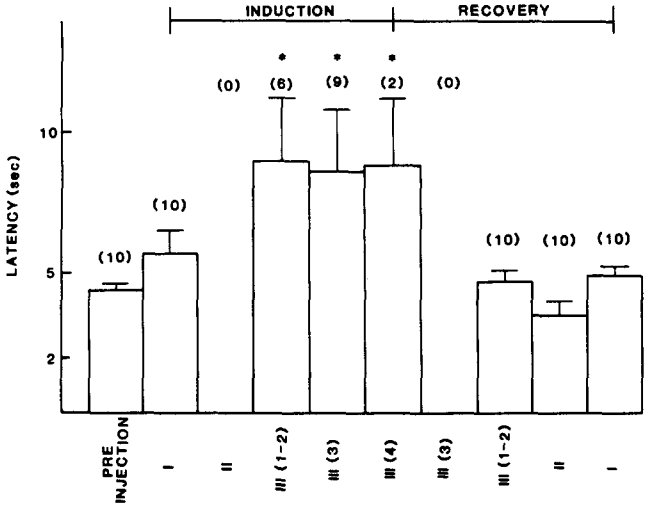

FIG. 7. Relationship between latency and level of anesthesia before and after IP injection of 65 mg/kg pentobarbital. Stage III of induction but not recovery was associated with increased latencies. The number in parentheses over a bar indicates the number of mice in each category.

tionships between latency and level of anesthesia or dose of pentobarbital. ANOVA revealed statistical significance for level of anesthesia, $F(5,40)=9.46$, $p<0.001$, and DMRT showed that stage III plane 4 was different from all other levels. No other level differed from any other. ANOVA also showed statistical significance for dose, $F(5,40)=8.61$, $p<0.001$, and DMRT showed that mice that had received 97.5 mg/kg of pentobarbital were different from those receiving any other dose; no other dose was different from any other doses.

Latency During Induction of and Recovery from Pentobarbital Anesthesia

All ten mice reached either stage III plane 3 or stage III plane 4 and were followed until complete recovery from anesthesia (termed stage I of the recovery phase). Some stages or planes were skipped by individual mice or lasted for such a short duration that mice could not be tested. For example, during the induction phase, no mice were tested in stage II (although all mice were tested in stage II during the recovery phase) or stage III plane 3 of the recovery phase (Fig. 7).

ANOVA showed a statistically significant effect: $F(7,59)=3.16$, $p<0.01$. DMRT showed that during the three periods when the animals were awake and showing no effects of pentobarbital (i.e., preinjection, stage I of induction, and stage I of recovery), latencies were not significantly different from each other (Fig. 7). These latencies were significantly different, however, from all stage III latencies, regardless of plane, during induction. The latency of stage III plane 1–2 of the recovery phase was not different from the preinjection latency but was different from the stage III plane 1–2 of the induction phase.

DISCUSSION

The results show that a dissociation can exist between the anesthetic and antinociceptive properties of compounds like ketamine and pentobarbital that is similar to the dissociation noted for inhalation anesthetics (4). We also found that mice may have increased (ketamine), decreased (urethane), or un-

changed (pentobarbital) responses to nociceptive stimuli in comparison with awake mice. These and other findings discussed below suggest that the anesthetized mouse may be a useful tool in the study of nociception.

An advantage of studying mice under general anesthesia is the decrease in the amount of statistical variation in the measurement of tail flick latency. When compared with mice treated with saline, the variability in latency among mice (interindividual variation) was reduced by induction of anesthesia with either urethane or ketamine, while the variation in latency for individual mice (intraindividual variation) was reduced by anesthesia induced with pentobarbital, urethane, or ketamine. Tail flick latency is a quick and simple technique for the measurement of nociception in rodents. Unfortunately, a major drawback is the high degree of variability that occurs among and within individual animals. This variability appears to be due in part to sensitivity to extraneous environmental cues that can result in transient alterations in nociception and obscure more subtle responses. Reduction of this variability by general anesthetics that limit sensory inputs but preserve latency responses may aid future studies.

The general anesthetics may also have a differential effect on endogenous factors important in nociception. As assessed with the use of morphine and naloxone, the effects of opiates, ordinarily affecting an animal's level of nociception, were minimized in mice anesthetized with pentobarbital and urethane. Others have found similar results with inhalation anesthetics (9). By contrast, mice anesthetized with a standard dose of ketamine were responsive to morphine. Other studies suggest that ketamine may work through opiate (6,10) as well as histaminergic, adrenergic, and cholinergic mechanisms (3, 11, 15). Thus, different anesthetics may have different effects on opiate action. If anesthetics can affect the action of exogenous opiates, they probably can alter the effects of endogenous opiates. This might be useful in the further study of nociception. For example, mice anesthetized with pentobarbital or urethane, in which the influence of opiate effects are minimal, could be used to study nonopiate mechanisms of analgesia.

The combination of a decrease in the influence of environmental cues and altered endogenous factors could explain the lack of correlation between the latencies obtained before and after induction of anesthesia. A correlation existed between the latencies obtained immediately before an injection of saline and those obtained ten min after injection. The correlation seemed weaker than in the experiment where the second latency was obtained immediately after the first without an interceding injection (r^2=.321 vs. .612). This illustrates the variability that is frequently seen in the measurement of tail flick latency in awake animals. The induction of anesthesia with pentobarbital or ketamine, however, totally abolished any correlation between pre- and postinjection latencies. Since mice anesthetized with urethane always achieved maximal latencies regardless of preanesthetic baselines, they, too, may be considered to have had a loss of preanesthetic influences. Such a loss of preanesthetic influences would eliminate another variable in the study of nociception in anesthetized mice.

In general, the level of anesthesia obtained with the dose of pentobarbital used in most of our experiments seemed to have little influence on latency (Fig. 6). However, the very deep levels of anesthesia not typically used in surgical procedures (stage III plane 3–4) that were induced by the large dose of 97.5 mg/kg pentobarbital did appear to increase latency. This probably was not due to a typical dose response relationship but to the toxicity of this nearly lethal dose. It also seemed that stage III plane 1–2 occurring during induction of anesthesia with pentobarbital may have been associated with a degree of analgesia not seen in stage III plane 1–2 occurring during recovery from the deeper stages. Species differences may also occur; it has been shown that low doses of pentobarbital decrease latency and potentiate the effects of morphine in rats (2). This raises the possibility that anesthetics may interact with other factors to modulate latency.

Taken as a whole, the results show, as measured by tail flick latency, that a dissociation can occur between a substance's anesthetic and analgesic properties at the doses typically used to induce general anesthesia, that latency time can vary with the anesthetic used, that anesthetics can differentially affect factors such as opiates that normally influence nociception, and that anesthetics can abolish the influence on latency of the preanesthetic state. The use of anesthetized animals—with their associated decreased statistical variability, decreased sensitivity to environmental and preanesthetic influences, facilitated experimental access for invasive procedures, and alteration of endogenous systems important in nociception—may be a useful tool in the study of nociception.

ACKNOWLEDGEMENTS

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