Displacement of Thiopental from Plasma Proteins by Nonsteroidal Anti-inflammatory Agents[1] (37387)

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Alteration of the distribution or metabolism of a barbiturate by a second drug can result in intensification and prolongation of its pharmacologic action. Thus, when sulfaethylthiazole was administered concurrently with pentobarbital or sodium salicylate, the concentrations of the unbound serum fractions of each drug were greater than those obtained when the two drugs were administered separately (1). Similarly, prior subcutaneous administrations of sulfaethylthiazole, sulfamethazine, sulfanilamide, salicylic acid, doxycline and *p*-aminosalicylic acid significantly increased pentobarbital sleeping time in mice (2). These findings were attributed to competition between these drugs and pentobarbital for serum proteins, resulting in an increase of unbound pentobarbital and consequently a higher concentration of pentobarbital in the brain. On the other hand, SKF 525-A has been shown to enhance barbiturate sleeping time by inhibiting the metabolic transformation of the barbiturate (3, 4).

We have examined several nonsteroidal anti-inflammatory agents for their capacity to alter the plasma binding of thiopental which, in some instances, caused reinduction of thiopental sleep. Agents tested were aspirin, phenylbutazone, indomethacin, mefenamic acid and naproxen,[2] a new potent anti-inflammatory, analgesic and antipyretic agent (5).

Materials and Methods. Male rats, body weight 135–165 g (Sprague–Dawley derived, Simonsen Lab. Gilroy, CA), and male New Zealand white rabbits, body weight 2.5–4 kg, were employed. Animals were acclimatized to laboratory conditions for at least 4 days before use. Food and water were allowed *ad libitum*.

Drugs were always administered in solution. Naproxen, indomethacin,[3] phenylbutazone,[3] mefenamic acid[3] and aspirin were dissolved in NaOH. Sodium thiopental was dissolved in normal saline. Sodium thiopental-2-^{14}C (sp act 5.19 mCi/mmole) was used without further purification.

Thiopental sleeping time was defined as the time elapsed between loss and regaining of the righting reflex.

Blood was collected in heparinized syringes by cardiac puncture, under light ether anesthesia when necessary, and plasma was obtained by centrifugation. For determination of radioactivity, plasma samples were mixed with 15 ml of the scintillation fluid previously described (6), and assayed by liquid scintillation spectrometry. All samples were corrected for quenching by external standardization.

Binding of thiopental to plasma proteins was studied by an ultrafiltration technique used extensively by others (7, 8). In the *in vitro* studies, thiopental-2-^{14}C, in a volume of 25 μl, was added to 5 ml of fresh, un-

[1] A portion of this study was presented at the ASPET Fall Meet., Stanford Univ., Aug. 25, 1970.

[2] The correct Chemical Abstracts index name for naproxen is (+)-6-methoxy-α-methyl-2-naphthaleneacetic acid but the compound was identified previously in the chemical and biological literature as *d*-2-(6′-methoxy-2′-napthyl) propionic acid.

[3] The authors are grateful to Geigy Pharmaceuticals, Ardsley, NY for generous supplies of phenylbutazone; to Merck and Company, Inc., West Point, PA, for indomethacin; and to Parke Davis and Company, Detroit, MI, for mefenamic acid.

diluted rabbit plasma and incubated at 37° for 15 min. The anti-inflammatory agent under study was then added (in a volume no larger than 200 μl) and the mixture was incubated for 30 additional min. The same volume of vehicle was added to control samples. In the *in vivo* studies, radioactive plasma samples were assayed for total thiopental equivalents and for extent of binding to plasma proteins. Dialysis tubing prepared from high purity, regenerated cellulose, with an average pore radius of 24 Å, was obtained from Union Carbide Corp., Chicago, IL. Tubing of 28 mm diameter was cut into 20 cm segments, soaked in distilled water for 30 min, blotted dry with tissue paper, and securely knotted at one end. Each bag was suspended in a 50 ml polypropylene centrifuge tube with the open end of the bag stretched over the lip of the tube and anchored in place with a plastic stopper. Plasma samples (2 ml) were added to the dialysis bag and centrifuged for 60 min at 1100*g* at room temperature (20–22°). This procedure resulted in 0.2–0.4 ml of ultrafiltrate. The method of Lowry *et al* (9), was used to confirm the absence of proteins in the ultrafiltrates.

Results and Discussion. In the experiments described in Table I, all rats received an iv dose of 25 mg/kg thiopental and the duration of loss of righting was determined. The average sleeping time of each group is listed in the column "initial sleep time." Immediately after regaining their righting ability from thiopental, the rats were given naproxen, aspirin or phenylbutazone iv at the indicated doses. Naproxen doses of 50, 200 and 400 mg/kg caused, respectively, 10, 40 and 100% of the rats to again lose their righting reflex. Likewise, 10, 60 and 80% of the animals again lost their righting reflex

TABLE I. Reinduction of Thiopental[a] Sleep in Rats by Nonsteroidal Anti-inflammatory Agents.[b]

Anti-inflammatory agent studied	Dose of anti-inflammatory agent[c] (mg/kg)	Rats re-sleeping (%)	Min		
			Initial sleep time	Latency period[d]	Resleep time[e]
Naproxen	0	0	30.0 ± 3.2	—	—
	50	10	47.2 ± 6.8	18	42
	100	50	22.0 ± 4.5	21.9 ± 4.8	29.9 ± 9.9
	200	40	29.6 ± 6.1	26.3 ± 7.3	54.3 ± 13.7
	300	90	21.2 ± 4.7	21.8 ± 7.0	104.2 ± 12.4
	400	100	22.9 ± 4.4	3.8 ± 1.3	103.7 ± 14.7
Aspirin	0	0	21.4 ± 3.7	—	—
	100	10	21.8 ± 4.4	1	2
	300	10	25.1 ± 3.9	1	8
	500	60	30.0 ± 3.2	1–2	10.8 ± 3.4
	700	80	18.6 ± 3.9	1–2	23.3 ± 6.5
Phenylbutazone	0	0	9.7 ± 2.2	—	—
	50	40	11.8 ± 2.1	<1	2.1 ± 0.7
	100	85	9.1 ± 1.9	<1	4.5 ± 0.7

[a] Thiopental given iv at a dose of 25 mg/kg induced sleep in all animals. The duration of sleep is indicated in the column "initial sleep time."

[b] All values are the mean ± SE of 10 rats except the phenylbutazone study, in which 20 animals were employed in each group.

[c] The anti-inflammatory agent under study was injected iv at the indicated doses immediately after the rats regained their righting reflex from thiopental.

[d] The "latency period" is that time between injection of the anti-inflammatory agent and loss of the righting reflex.

[e] The "resleep time" is that time the animals slept after the dose of the anti-inflammatory agent.

after the administration of 300, 500 and 700 mg/kg, respectively, of aspirin. Employing phenylbutazone at doses of 50 and 100 mg/kg caused 40 and 85% of the rats to lose their righting ability.

The ability of naproxen, aspirin and phenylbutazone to cause rats to again lose their righting ability when administered iv at the time of righting from thiopental is probably not due to any central nervous system depressant property of the anti-inflammatory agents. None of these agents, when administered alone to rats at the doses employed in this study, caused the animals to lose their righting ability.

When aspirin or phenylbutazone was employed, animals lost their righting ability within 1–2 min. In the rat studies employing naproxen, the animals lost their righting ability within 3–4 min after administration of 400 mg/kg naproxen but at lower doses the latency period was approximately 20 min. This long latency period observed with naproxen is not understood but it may be related to the fact that naproxen has some central nervous system stimulating properties in the rat (5). It is possible that at the highest dose of naproxen employed (400 mg/kg), the displacement of thiopental is so rapid and extensive that it quickly overcomes any central nervous system stimulating action of naproxen and results in a short latency period. Another possibility is that thiopental is displaced from other body compartments as well as from plasma proteins.

Mefenamic acid and indomethacin could not be tested for their ability to reinduce thiopental sleep because of their acute toxicity to rats. Intravenous administration of mefenamic acid (100 mg/kg) and indomethacin (50 mg/kg) caused death in all animals. Nonlethal doses of either agent, when given to rats immediately after their righting from thiopental, did not cause reinduction of thiopental sleep.

Displacement of thiopental from plasma proteins by the nonsteroidal anti-inflammatory agents might be the basis for reinduction of thiopental sleep since all of these agents are strongly bound to plasma proteins. In all species examined, naproxen, in concentrations less than 100 μg/ml, is 98–99.9% bound to plasma proteins (10). Accordingly, the ability of naproxen to displace thiopental from plasma proteins *in vivo* was examined. Rabbits were employed since multiple blood samples of adequate size could be obtained in each *in vivo* experiment. In the experiment depicted in Fig. 1A, rabbits were given thio-

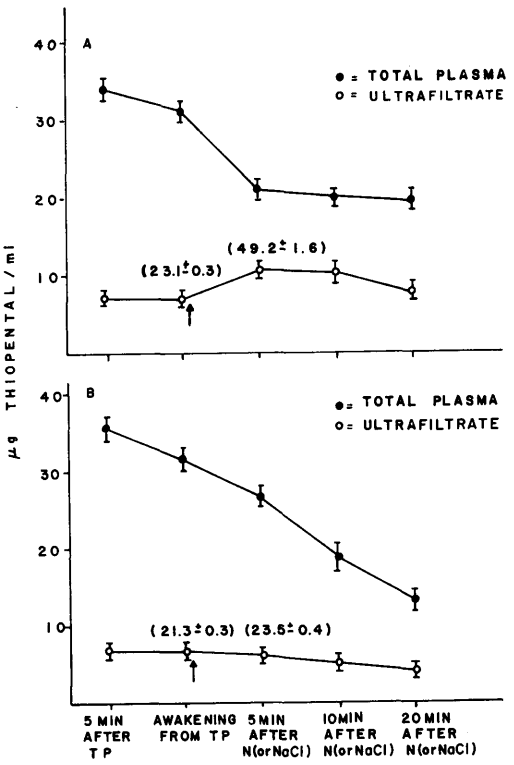

FIG. 1. Ability of naproxen (N) to displace thiopental (TP) from plasma proteins of the rabbit *in vivo*. All animals were dosed iv with TP (20 mg/kg). In each experiment, five blood samples were obtained and assayed for total TP in plasma and for unbound TP: (i) 5 min after injection of TP, (ii) upon awakening from TP, (iii) 5 min after injection of N (or NaCl), (iv) 10 min after injection of N (or NaCl) and (v) 20 min after injection of N (or NaCl). (A) The arrow indicates time of intravenous injection of N (200 mg/kg). Each point represents the mean ± SE of six rabbits. (B) The arrow indicates time of injection of NaCl (5 ml of 0.45 *M*). Each point represents the mean ± SE of four rabbits. In both (A) and (B), the numbers in parentheses indicate the percentage of free thiopental.

pental iv at a dose of 20 mg/kg after which the animals slept approximately 12 min. When 200 mg/kg of naproxen was injected iv immediately after the animals regained their righting reflex from the dose of thiopental, the rabbits again lost their righting reflex within 1–4 min and remained asleep for an average of 8 min. The data in Fig. 1 demonstrate quite clearly that naproxen displaced thiopental from plasma proteins of the rabbit under the experimental conditions employed. The concentration of free thiopental 5 min after naproxen administration was 10.2 μg/ml, which is significantly higher than the concentration seen upon waking from the dose of thiopental ($p < 0.05$). Thus, after naproxen, about 49% of the thiopental was found to be not bound to plasma proteins whereas only 23% was not bound at the time of regaining the righting reflex from the thiopental dose. Furthermore, the concentration of free thiopental in plasma 5 min after injection of naproxen is also significantly higher ($p < 0.05$) than the concentration of free thiopental found in plasma 5 min after injection of a NaCl solution (Fig. 1B).

Figure 1B shows that the injection of 5 ml of 0.45 *M* NaCl (same volume and molarity as naproxen injections) immediately upon regaining the righting reflex has no effect on the binding of thiopental to plasma proteins. Rabbits did not lose their righting reflex after injection of NaCl. The curve depicted is identical to one obtained when total and free thiopental are determined after a single iv dose of thiopental.

The data presented in Table II indicate that high concentrations of naproxen, aspirin, mefenamic acid, indomethacin and phenylbutazone displaced thiopental from plasma proteins *in vitro*. Employing a concentration of 5.3 m*M*, all agents displaced thiopental; however, at a concentration 10 times lower, none of the anti-inflammatory agents displaced thiopental from plasma proteins of the rabbit.

From the data presented in Table I, it is apparent that naproxen, phenylbutazone and aspirin reinduced thiopental sleep in rats in a dose related manner. Furthermore, when naproxen was employed, the larger the

TABLE II. Ability of Nonsteroidal Anti-inflammatory Drugs to Displace Thiopental from Plasma Proteins of the Rabbit *in Vitro*.[a]

Anti-inflammatory drug	Concn (m*M*)	Thiopental concn in ultrafiltrate (μg/ml)	Free thiopental (%)
None	—	5.13 ± 0.42	18.9 ± 1.5
Naproxen	5.3	8.24 ± 0.34[b]	30.8 ± 1.8[b]
	0.53	4.65 ± 0.39	17.0 ± 0.9
Aspirin	5.3	7.87 ± 0.93[b]	30.3 ± 3.8[b]
	0.53	5.15 ± 0.43	19.8 ± 1.8
Mefenamic acid	5.3	9.58 ± 0.45[b]	34.5 ± 1.4[b]
	0.53	5.07 ± 0.45	18.6 ± 1.2
Indomethacin	5.3	9.88 ± 0.45[b]	36.3 ± 1.2[b]
	0.53	5.94 ± 0.67	21.6 ± 2.3
Phenylbutazone	5.3	11.70 ± 0.89[b]	40.3 ± 3.3[b]
	0.53	5.54 ± 0.28	19.7 ± 1.3

[a] Sodium thiopental was added to fresh rabbit plasma (heparinized) to a concentration of 26.1 to 29.0 μg/ml (calculated as the free acid). After incubation at 37° for 15 min, the anti-inflammatory drug was added and the mixture was incubated an additional 30 min (control samples were incubated for 45 min). Suitable samples were then centrifuged for 60 min at 1100*g* at 20–22° and the concentration of thiopental in the ultrafiltrates was determined. The values represent the mean ± SE of three separate experiments.

[b] $p < 0.001$, significantly different from the value obtained when no anti-inflammatory drug was added to plasma.

dose of naproxen the longer the rats slept, a finding compatible with inhibition of the metabolism of thiopental by naproxen. The notion that naproxen inhibits the metabolism of thiopental is further supported by the rabbit study in which the slope of decay of total thiopental was less steep after naproxen (Fig. 1). Another possible explanation for these observations is that naproxen displaced thiopental from other body compartments.

Summary. Intravenous administration of high doses of aspirin, phenylbutazone or naproxen to rats which had just regained the righting reflex from an anesthetic dose of thiopental caused them to again lose their righting reflex. Naproxen was also shown to reinduce thiopental sleep in rabbits and the reinduction of sleep was correlated with a marked elevation in the concentration of unbound thiopental in plasma. Furthermore, high concentrations of aspirin, phenylbutazone, indomethacin, mefenamic acid and naproxen were shown to displace thiopental from rabbit plasma proteins *in vitro*. These results suggest that high concentrations of nonsteroidal anti-inflammatory agents, all of which strongly bind to plasma proteins, effectively displace thiopental from plasma proteins, thus leading to reinduction of its action.

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