Anesthesiology
62:11-16, 1985

Increased Hepatic Microsomal Enzyme Activity after Surgery under Halothane or Spinal Anesthesia

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Thirty-two fit patients scheduled for explorative arthrotomy of the knee were allocated randomly to either halothane/oxygen anesthesia or spinal anesthesia with bupivacaine 0.25 mg · kg^{-1}. The day before and 1, 10, and 21 days after surgery, the aminopyrine breath test (ABT) was performed. The day before and 5, 10, and 21 days after surgery, the antipyrine clearance (AP_{cl}) was measured by the single sample saliva technique. The ABT as well as the AP_{cl} were increased significantly postoperatively ($P < 0.01$). The day after surgery the ABT was increased by 13 ± 21% in the spinal anesthesia group only, whereas a late increase by 14 ± 31% was found in the halothane group. Five days after surgery, the AP_{cl} was increased by 36 ± 45% in the spinal anesthesia group and by 21 ± 28% in the halothane group. Both tests returned to base line values within 3 weeks postoperatively. In five volunteers following the same sampling scheme but receiving bupivacaine 0.25 mg · kg^{-1} im without surgery, no change in the ABT or the AP_{cl} was observed. The authors conclude that surgery may cause microsomal enzyme induction regardless of the anesthetic agent or technique used. The mechanism of this induction remains to be elucidated. (Key words: Anesthetic techniques: spinal. Anesthetics, local: bupivacaine. Anesthetics, volatile: halothane. Biotransformation (Drug). Enzymes: induction. Metabolism: microsomes.)

INCREASED HEPATIC microsomal enzyme activity, as assessed by antipyrine metabolism, has been reported after surgery lasting about an hour under various regimens of general anesthesia including halothane.[1-4] However, extensive and long-lasting (4 h or more) surgical procedures seem to lead to depressed enzyme activity.[2] It is not known whether these alterations in microsomal enzyme activity are related to general anesthesia *per se* or to other factors associated with surgery. Neither have the effects of regional anesthesia been reported except for observations in three patients suggesting no difference from general anesthesia.[1]

Prolonged experimental enflurane anesthesia[5] and occupational exposure to waste halothane[6] increase the metabolism of model compounds used as indices of microsomal activity. Results from animal investigations are conflicting but do indicate that single or repeated exposure to ethers such as diethyl ether induces microsomal enzyme activity to the same extent as phenobarbital,[7,8] whereas nonethers including halothane have less[7] or no[8] inducing capacity.

In order to investigate whether postsurgical enzyme induction is related to surgery or the employed anesthetics we performed a randomized controlled study of surgery under general anesthesia or regional anesthesia, the latter requiring only a small amount of local anesthetic, which would be expected to be nearly inert. Two of the most widely used indices of microsomal enzyme activity, antipyrine and aminopyrine metabolism, were measured before and after surgery, lasting less than 1 h in subjects who received either halothane/oxygen or spinal anesthesia.

Material and Methods

Thirty-two fit patients ages 18-50 yr and scheduled for explorative arthrotomy of the knee for suspected meniscal injuries participated after giving informed consent. The investigation was approved by the local ethical committee of Copenhagen County. The patients were requested to sustain their individual dietary and smoking habits throughout the study.

All patients were premedicated with meperidine 1 mg · kg^{-1} im and were allocated at random to either halothane (17 patients) or spinal anesthesia (15 patients). Halothane was administered in oxygen via a face mask. The patients breathed spontaneously. Spinal anesthesia was achieved by subarachnoid administration of bupivacaine 0.5% 0.25 mg · kg^{-1} between L_3 and L_4. Both regimens were supplemented with meperidine iv as needed. Meperidine im and later acetaminophen po were used as needed for postoperative pain relief. Except for oral contraceptives taken by two patients, one in each group, no other drugs were administered.

The day before and 1, 10, and 21 days after surgery, the 2-h aminopyrine breath test[9] was performed at the hospital. After an overnight fast, the patients ingested 2 μCi ^{14}C-aminopyrine. Two hours later, carbon dioxide was collected by exhalation into a scintillation vial containing hyamine hydrocloride-ethanol and thy-

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Received from the Department of Medicine F, Orthopedics T_3, and Anaesthesia[3], Gentofte Hospital; Department of Organic Chemistry, Royal Danish School of Pharmacy, Denmark. Accepted for publication June 15, 1984. Part of the material was presented at the Second World Conference on Clinical Pharmacology and Therapeutics, Washington DC, August 1983.

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TABLE 1. Personal Data from 32 Patients Undergoing Surgery of the Knee under Halothane Anesthesia (Halothane Group) or Spinal Anesthesia (Spinal Group) and Five Volunteers (Control Group) Receiving Local Anesthetic without Surgery (Values Are Median [Range])

	Age (yr)	Sex (male/female)	Body Weight (kg)	Body Height (cm)	Daily Coffee/Tea Consumption (Index*)	Daily Alcohol Consumption (Drinks)	Daily Cigarette Consumption (n)
Halothane group (n = 17)	32 (19–50)	10/7	73 (47–100)	174 (152–190)	6 (10–11)	1 (0–3)	7 (0–30)
Spinal group (n = 15)	36 (18–47)	10/5	72 (45–87)	175 (162–189)	5 (2–10)	1 (0–3)	5 (0–20)
Control group (n = 5)	30 (27–36)	4/1	68 (60–75)	182 (180–186)	6 (3–10)	1 (0–3)	1 (0–3)

* Coffee/tea index = no. of cups of coffee + 0.6 times no. of cups of tea.

molphthalein. A shift from blue to colorless indicated trapping of exactly 2 mmol CO_2. A scintillation cocktail was added and the specific activity was measured in a liquid scintillation counter, adjusting for quench by external standardization. The cumulative ^{14}C-output from ingestion to sampling was estimated by multiplying the sample activity by the endogenous CO_2 output, which was estimated to be 9 mmol · kg^{-1} · h^{-1}.[9] The percentage of administered ^{14}C that was excreted in the breath as $^{14}CO_2$ in 2 h (ABT) was used for the quantitative assessments of aminopyrine metabolism.

The day before surgery and 5, 10, and 21 days postoperatively, antipyrine clearance was estimated. After an overnight fast, the patient ingested 1 g antipyrine dissolved in 50 ml juice. Twenty-four hours later, 5 ml of saliva was collected. The concentration of antipyrine was measured by high-pressure liquid chromatography. The system included a fixed wavelength UV detector (254 nm) and was fitted with a Spherisorb® ODS (5 μm) column. Saliva samples were mixed with perchloric acid containing the internal standard, fenacetin, and the supernatant after centrifugation was injected. The eluent was methanol/water. The antipyrine clearance was calculated from the equation $AP_{cl} = \frac{\ln (D/V_D) - \ln c_t}{t} \times V_D$, where D is the administered dose of antipyrine; V_D is the apparent volume of distribution estimated from sex, age, body weight, and height; t is the time of sampling; and c_t is the corresponding concentration in saliva.[10] In addition to the 32 patients, five healthy volunteers were studied using the same sampling scheme, but following administration of bupivacaine 0.25 mg · kg^{-1} im without surgery.

The time course of the measurements within a group or a subgroup was treated statistically as a randomized block experiment. If two-way analysis of variance was significant, differences between means were tested with Scheffe's simultaneous confidence interval procedure. Comparison of relative changes from presurgery values between groups or subgroups was done statistically as a completely randomized experiment design. If one-way analysis of variance of the means of the relative change from base line value of all the postsurgery measures in the two groups or subgroups in question was significant, the differences between means of parallel measures were compared with Bonferroni's test. $P < 0.05$ was considered statistically significant.

Results

Seventeen patients received halothane anesthesia lasting 51.5 ± 18.4 min (mean ± SD), and 15 patients received spinal anesthesia. One patient from the halothane group did not complete the antipyrine clearance measurements. The groups subjected to surgery were similar as regards the recorded clinical and personal data (table 1).

The aminopyrine breath test values increased significantly after surgery in each of the anesthesia groups ($P < 0.01$) (fig. 1 and table 2). In the spinal anesthesia group the aminopyrine breath test was increased only on the day after surgery ($P < 0.05$). Only at 10 days postoperatively did a similar increase occur in the halothane anesthesia group ($P < 0.05$). However, the relative change from the base line value in aminopyrine breath test in the two groups subjected to surgery did not differ significantly at any measurement ($P > 0.05$).

The antipyrine clearance was increased significantly at 5 days postoperatively in each of the groups subjected to surgery ($P < 0.01$) (fig. 2, table 3). It appears that the male subjects were responsible for the major part of the postoperative increase in the antipyrine clearance ($P < 0.05$). However, there was no significant sex-related difference in the aminopyrine breath test ($P > 0.1$).

Neither the aminopyrine breath test nor the antipyrine clearance changed significantly in the control group ($P > 0.12$; two-way analysis of variance). The measured antipyrine clearance and the aminopyrine breath test correlated significantly before ($r = 0.72$,

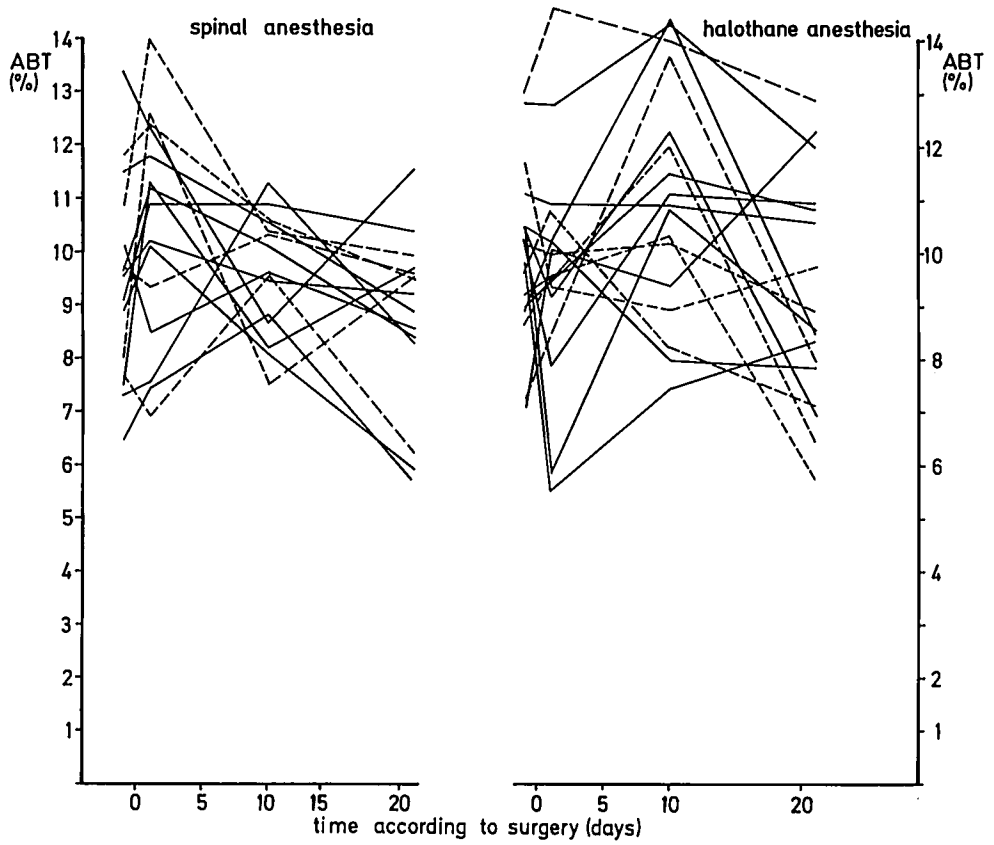

FIG. 1. Aminopyrine breath test (%) in 32 fit patients before and after surgery of the knee under halothane or spinal anesthesia. There was no significant difference between the male (solid lines) and the female subjects (broken lines).

$P < 0.001$) and 3 weeks after ($r = 0.60$, $P < 0.001$) surgery, whereas the correlation between the tests performed the tenth postoperative day was insignificant ($r = 0.24$, $P > 0.1$).

Discussion

Before and after surgery under halothane or spinal anesthesia we have investigated microsomal enzyme

TABLE 2. Aminopyrine Breath Test (ABT%) Measured before and Three Times after Surgery under Halothane or Spinal Anesthesia and in Controls Receiving Local Anesthetic without Surgery (The Percentage Deviation from the Base Line Value is in Brackets. Values are given as mean ± SD.

	No. of Days before (−) or after (+) Surgery			
	−1	+1	+10	+21
Halothane anesthesia				
Males n = 10	9.98 ± 1.50	9.24 ± 1.97 (−6 ± 26)	11.1 ± 2.44 (12 ± 26)	9.72 ± 1.86 (−1 ± 26)
Females n = 7	9.92 ± 1.79	10.5 ± 4.11 (6 ± 13)	11.1 ± 2.08 (16 ± 36)	8.47 ± 2.20 (−15 ± 15)
Males + females n = 17	9.95 ± 1.59	9.75 ± 2.19 (1 ± 21)	11.1 ± 2.20 (14 ± 31)*	9.18 ± 2.07 (−6 ± 17)
Spinal anesthesia				
Males n = 10	9.35 ± 1.94	10.2 ± 1.57 (11 ± 18)	9.54 ± 1.11 (6 ± 24)	8.67 ± 1.72 (−6 ± 18)
Females n = 5	9.61 ± 1.61	11.1 ± 2.57 (14 ± 23)	9.69 ± 1.15 (2 ± 13)	9.00 ± 1.34 (−5 ± 14)
Males + females n = 15	9.43 ± 1.84	10.5 ± 2.00 (13 ± 21)*	9.60 ± 1.13 (6 ± 21)	8.77 ± 1.62 (−6 ± 17)
Controls n = 5	7.75 ± 0.20	7.41 ± 0.77 (−4 ± 7)	7.35 ± 0.80 (−3 ± 14)	8.21 ± 1.00 (5 ± 13)

* $P < 0.05$ *versus* preceding and subsequent measurements.

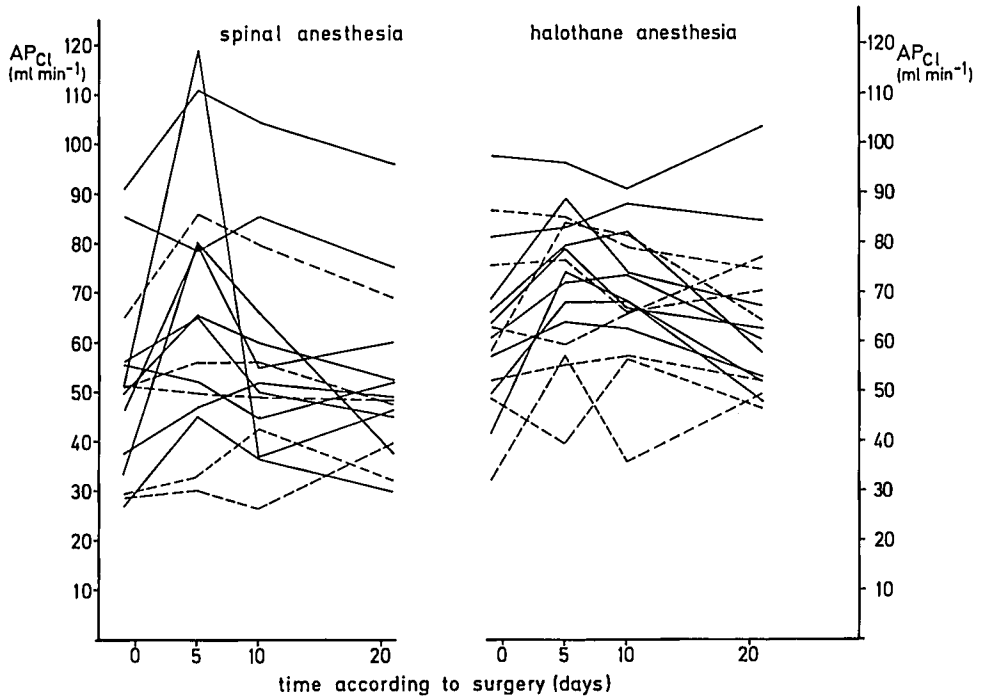

FIG. 2. Antipyrine clearance (ml · min^{-1}) in 31 fit patients before and after surgery of the knee under halothane or spinal anesthesia. The increase in the male subjects (solid lines) was significantly different from that found in the female subjects (broken lines) ($P < 0.05$).

activity as assessed by two of the most widely accepted methods, the aminopyrine breath test[9] and antipyrine clearance.[11] The two tests were done simultaneously at three of the four occasions. Antipyrine clearance was not measured on the day after surgery due to the risk of selfinduction of antipyrine metabolism, whereas the aminopyrine breath test was omitted at day 5 postsurgery because of ethical problems with the use of radioactive material outside the hospital.

The aminopyrine breath test has not been used previously for assessment of enzyme induction after surgery. We observed a small increase on the day after surgery in our regional (spinal) and 10 days after surgery in our general (halothane) anesthesia group. The increase in antipyrine clearance that we observed after surgery was of the same magnitude as that reported in earlier studies following surgery of similar duration (½–2 h) using various regimens of general anes-

TABLE 3. Antipyrine Clearance (AP_{Cl}, ml · min^{-1}) Measured before and Three Times after Surgery under Halothane or Spinal Anesthesia and in Controls Receiving Local Anesthetic without Surgery (The Percentage Deviation from the Base Line Value is in Brackets. Values Are Given as Mean ± SD.)

	No. of Days before (−) and after (+) Surgery			
	−1	+5	+10	+21
Halothane anesthesia				
Males n = 9	62.5 ± 11.6	76.0 ± 7.2 (27 ± 24)*	72.8 ± 8.9 (22 ± 23)	62.5 ± 11.8 (1 ± 11)
Females n = 7	62.4 ± 20.9	68.1 ± 18.9 (15 ± 32)	67.1 ± 17.7 (11 ± 16)	67.9 ± 20.1 (13 ± 21)
Males + females n = 16	62.5 ± 16.6	72.6 ± 13.9 (21 ± 28)*†	70.3 ± 13.8 (17 ± 21)	64.6 ± 15.8 (6 ± 17)
Spinal anesthesia				
Males n = 10	56.0 ± 17.8	74.2 ± 23.7 (48 ± 49)*	59.1 ± 20.2 (16 ± 34)	54.7 ± 18.0 (3 ± 17)
Females n = 5	45.0 ± 14.2	50.9 ± 20.1 (11 ± 12)	50.7 ± 17.4 (13 ± 20)	47.3 ± 12.4 (8 ± 16)
Males + females n = 15	50.7 ± 18.8	66.4 ± 25.1 (36 ± 45)*†	56.3 ± 19.7 (15 ± 32)	51.9 ± 16.7 (5 ± 17)
Controls n = 5	62.0 ± 19.6	56.2 ± 14.5 (−8 ± 14)	55.9 ± 15.6 (−9 ± 1)	60.6 ± 21.5 (−4 ± 9)

* $P < 0.01$ *versus* initial value.

† $P < 0.05$ *versus* control group.

thesia[1-4] and it occurred in both our general (halothane) and our regional (spinal) anesthesia groups. In order to administer high concentrations of halothane, which has shown enzyme-inducing properties in prolonged occupational exposure,[6] we had even omitted nitrous oxide and barbiturate from the general anesthesia. Thus, general anesthesia *per se* does not seem to be responsible for the signs of microsomal enzyme induction observed after surgery.

The single sample saliva technique[10] employed for estimation of antipyrine clearance in this study is dependent on accurate recording of the sampling time. However, earlier studies have shown that unskilled workers are able to perform the test correctly at home.[12] Accordingly, a variation in the antipyrine clearance observed after surgery of the same magnitude as in our study has been reported by authors using multiple samples.[2,3] Moreover, a computer simulation study has demonstrated that changes in the volume of distribution (V_d) have very little effect on the antipyrine clearance measured by the single sample technique.[13]

A correlation between the aminopyrine breath test and the antipyrine clearance as poor as that observed 10 days after surgery in our study has been observed in patients receiving anticonvulsants.[14] Thus, an enzyme-inducing impact may act differently on aminopyrine and antipyrine metabolism, thereby probably reflecting differences in the susceptibility of isozymes of cytochrome P-450. It is believed that from 10 to 1,000 genetically controlled isozymes exist and, although greatly overlapping, each isozyme is responsible for the metabolism of a limited number of compounds.[15]

The sex-related difference in the changes in the antipyrine clearance after surgery has not been reported previously. Ninno *et al.*[4] found a substantial increase in the antipyrine clearance a week after hysterectomy under halothane/nitrous oxide/oxygen anesthesia. However, hysterectomy may have a different impact on the microsomal enzyme system than arthrotomy of the knee. In the rat, enzyme induction with, *e.g.*, phenobarbital, is partly androgen dependent,[16] whereas only very small sex-related differences in the antipyrine metabolism are known to occur in humans.[17]

The antipyrine clearance and aminopyrine breath test were unchanged in the control group receiving local anesthesia without surgery, indicating that neither the administered amount of bupivacaine nor the repeated ingestion of aminopyrine and antipyrine are responsible for the microsomal enzyme induction observed in the patients subjected to surgery. The other prescribed drugs, meperidine and acetaminophen probably have no enzyme inducing properties.[11] Moreover, the patients were asked to keep their dietary, smoking, and drug habits constant throughout the study. Bedrest, fluid restriction, and starvation were minimal as the patients were discharged 24 h after surgery and followed as outpatients.

Surgery and trauma usually are followed by profound hormonal and metabolic changes. An increased *in vitro* activity of some microsomal enzymes has been reported in rats subjected to stress of several days duration,[18,19] although no alteration in the *in vivo* metabolism of antipyrine was observed. A similar increase in the *in vitro* enzyme activity in the rat has been reported after prednisolone treatment.[20] However, administration of hydrocortisone to humans in amounts mimicking the cortisol part of the endocrine stress response failed to alter antipyrine metabolism.[21] Short-term (2.5 h) stress, such as hind limb ligation, simulating surgery, has been found to decrease rat hexobarbital sleeping time and increase plasma clearance of the drug.[22] This effect could be abolished by removal of the adrenals or pituitary before application of the stress.[23] A decreased antipyrine elimination has been observed after major and long-lasting (more than 4 h) abdominal surgery,[2] implying at least an equally strong stress response. This, in turn, might be attributed to a deteriorated general condition of the patients after surgery.[24] Accordingly, no particular factor related to surgery can be identified yet as a likely mediator of the observed increase in aminopyrine and antipyrine metabolism.

The average increase in antipyrine clearance was rather small (21–37%) and rarely would be of clinical importance. However, the changes showed great variation, and in a few patients increases exceeding 100% were observed, suggesting clinically significant changes in the metabolism of other drugs.

In conclusion, we have demonstrated an increase in antipyrine and aminopyrine metabolism, indicating induction of the hepatic microsomal enzyme system after surgery of the knee under halothane as well as spinal anesthesia. Factors other than anesthetic agents seem to be responsible.

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