# Methyleugenol as a surgical anesthetic in rodents

E. A. Carlini, K. Dallmeier and J. L. Zelger

*Departamento de Psicobiologia, Escola Paulista de Medicina, Rua Botucatu, 862, 04023 São Paulo (Brasil), 8 September 1980*

*Summary.* Methyleugenol, suspended in Tween-80 or cremophor EL, at the dose 200–275 mg/kg i.p., is a safe anesthetic agent in rats and mice submitted to surgical procedures in the brain, without some of the inconvenience of sodium pentobarbital.

The ideal anesthetic agent for laboratory animals is still waiting to be discovered. Pentobarbital anesthesia through the i.p. route is far from convenient. A 40–50-mg/kg dose can kill a few animals and be insufficient to induce anesthesia in others; the induction of bronchial secretion is another handicap, and the fact that it is a scheduled drug causes time-consuming difficulties with supply and control. Ether anesthesia is also inconvenient. Besides being volatile, it is a very inflammable substance, and it has to be administered continuously or repeatedly. In order to overcome these difficulties, Valenstein[4] recommended as anesthetic agent the administration of 2 drugs, sodium pentobarbital and chloral hydrate. Although this mixture seems to induce an efficient surgical anesthesia[2], the experimenter has to prepare 2 solutions and to inject them separately; furthermore, anesthesia is attained only about 5 min after the 2nd injection.

In previous work in our laboratory[3] we reported that methyleugenol induced a loss of the righting reflex of rats and mice and changed the rat's EEG in a very similar way to sodium pentobarbital.

We want now to report that methyleugenol is being used routinely in our laboratory to anesthetize rats and mice with good results. Initially, we calculated the $ED_{50}$ for loss of the righting reflex. Groups of 6 male Wistar rats, 2.5 months old, weighing around 250 g, received progressively increasing doses of mehyleugenol, starting with 50 mg/kg, i.p. The drug (obtained from Pfaltz & Bauer, Inc.) was first thoroughly mixed with cremophor EL (1.0% in final suspension) and further diluted with distilled water to obtain the desirable final concentrations of methyleugenol (250 mg/ml for rats; 25 mg/ml for mice); the milky suspensions obtained were stable for at least 2 weeks. The $ED_{50}$, calculated according to Litchfield & Wilcoxon[1], was 70 mg/kg (95% fiducial limits, 56.5–86.6). Next, starting with a dosage of 275 mg/kg, we proceed to calculate the $LD_{50}$ for rats; the value found within 24 h was 470 mg/kg (95% fiducial limits, 391–564).

Based on these 2 values and also on previous data[3], groups of 6 male rats, 3 months old, received either 250 or 275 mg/kg of methyleugenol suspended in cremophor EL. Injections were performed with suspensions which were either freshly prepared or had been kept for 2 weeks at room temperature (air conditioned laboratory at 23±2 °C) in dark flasks. The latency of losing and regaining the righting reflex, and the latency of becoming insensitive to tail pinching and the duration of this insensibility were recorded. The rats lost the righting reflex in about 140 sec and became completely insensitive to pinching of the tail in about 4–5 min. The duration of tail insensitivity was slightly less than 1 h and after 1.5–2 h all animals regained the righting reflex. No deaths occurred and all animals were apparently in good health 1 week later. The results were the same regardless of whether or not the suspension was freshly prepared, showing that it is stable for at least 2 weeks. It was also noticed, confirming a previous report[3], that anesthesia was not preceeded by an excitation phase as happens with pentobarbital, and the animals presented marked abdominal muscle flaccidity.

Table 1 shows the results obtained when 200, 250 and 300 mg/kg of methyleugenol suspended with the help of Tween-80 (1.0% in final suspension) was injected in groups of 10 rats or mice. The results were very similar to those of the previous experiment (with cremophor EL), although one rat died of respiratory arrest after 250 mg/kg. All the other rats were in good health 1 week later. Table 1 shows

Table 1. Effects of methyleugenol suspended in water with 1% of Tween-80; i.p. injections in rats and mice

| Animal | 200 mg/kg | | | | 250 mg/kg | | | | 300 mg/kg | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Loss of righting reflex | | Tail pinching insensibility | | Loss of righting reflex | | Tail pinching insensibility | | Loss of righting reflex | | Tail pinching insensibility | |
| | Latency (sec) | Duration (min) | Latency (sec) | Duration (min) | Latency (sec) | Duration (min) | Latency (sec) | Duration (min) | Latency (sec) | Duration (min) | Latency (sec) | Duration (min) |
| Rat | 121±23 | 62 ±21 | 331±29 | 15±9 | 129±29 | 144±32 | 305±93 | 63±25 | – | – | – | – |
| Mouse | 92±19 | 7.5± 5.3 | 126±37 | – | 88±24 | 12±11 | 158±19 | – | 61±15 | 38±29 | 95±24 | 20±12 |

Table 2. Anesthesia induced by sodium pentobarbital and methyleugenol in two central nervous system surgeries

| Surgical procedure | Anesthetic (mg/kg) | Rat Sex | Age (months) | Weight (g) | Duration of surgery (min) | Deaths during and shorthly after surgery (no. deaths/no. operated) | Presence of bronchial secretion |
|---|---|---|---|---|---|---|---|
| Lesion of substantia nigra | Sodium pentobarbital (30–40) | Female | 2 | 150± 5 | 20–25 | 7/15 | 15/15 |
| | Methyleugenol (250) | Female | 2 | 152± 6 | 20–25 | 0/15 | 0/15 |
| Electrod implantations | Methyleugenol (275–325) | Male | 2.5 | 248±10 | 60–90 | 1/23* | 0/23 |

*A 2nd animal died 2 days later of infection.

also that mice responded less to methyleugenol. Thus, with 300 mg/kg they had about 20 min of tail insensibility and 38 min of righting reflex loss. Nevertheless, during this time period the mice could be submitted to surgical procedures in the abdomen; surgery in the brain was not tried.

Finally, table 2 gives the results employing methyleugenol suspended with cremophor EL in 2 surgical procedures. 2-month-old female reats were stereotaxically operated for unilateral destruction of substantia nigra through electrolysis. With sodium pentobarbital 7 out of 15 animals died and all of them presented bronchial secretion; it was difficult to find an optimal dose, as with 30 mg/kg most females still reacted to surgical manipulations whereas 40 mg/kg was too much for several of them. Conversely, no such problems occurred under 250 mg/kg of methyleugenol. All animals recovered well within 90–120 min after injection and no deaths or signs of sickness were noticed 1 week later. The 2nd surgical procedure, implantation of 7 electrodes for EEG and EMG recordings, took about 90 min to complete. 275 mg/kg of methyleugenol yielded a good degree of anesthesia although for a few animals a supplementary dose of 50 mg/kg was necessary after the 60–70th min. Even with this larger dose only 1 animal was lost during surgery; a 2nd animal died of infection 2 days later. It is worth mentioning, too, that soon after losing the righting reflex (about 2 min) the animal can be easily handled for the preparatory acts of surgery such as shaving the head and placement in the stereotaxic apparatus. This can save the time elapsing between loss of the postural reflex and the onset of insensitiveness (table 1).

In summary, our data show that methyleugenol can be an useful anesthetic agent for surgery in rats and mice. It is a cheap drug, well tolerated by the animals, it is easily available as its use is not restricted by law, and suspensions of it, which are stable for at least 2 weeks, can be prepared with the help of 2 easily available inert substances, cremophor EL and Tween-80.

1 J.T. Litchfield, Jr, and F. Wilcoxon, J. Pharmac. exp. Ther. *96*, 99 (1949).
2 R.E. Musty, C.J. Lindsey and E.A. Carlini, Psychopharmacology *48*, 175 (1976).
3 A.B. Sell, E.A. Carlini, Pharmacology *14*, 367 (1976).
4 E.S. Valenstein, J. exp. Analysis Behav. *4*, 6 (1961).

## Effect of acetylsalicylic acid on iron absorption in the rat[1,2]

P. Glikman, A. Gutnisky, M.F. Gimeno and A.L. Gimeno

*Centro de Estudios Farmacológicos y de Principios Naturales (CEFAPRIN), and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), V. de Obligado 2490, 1428 Buenos Aires (Argentina), 3 June 1980*

*Summary.* The in vivo administration of $^{59}Fe$ to the rat accompanied by acetylsalicylic acid (ASA) enhanced significantly counts in blood, spleen,liver and femur without affecting those of the intestine. The results suggest that ASA augments iron absorption either via an inhibitory action on the synthesis of prostaglandins or by a purely chemical mechanism.

Several factors can influence intestinal iron absorption[3]. One, operative at the level of the intraluminal compartment, is related to the different forms of iron intake as well as with the simultaneity of food administration[4-8]. Another relevant influence is the intrinsic ability of the intestinal mucosa to absorb iron[9-11]. In addition the total body iron requirements are also important for the maintenance of a normal erythropoiesis[12,13]. In previous studies with isolated rat intestine we have documented that prostaglandins $E_1$ and $E_2$ can influence the mechanism of intestinal iron absorption, acting in a double fashion: a) on the uptake of iron by the villous cells of the intestine and b) on the processes by which the iron is transported at the serosal end of the intestinal barrier[14]. Based on these results it was decided to study the effect of acetylsalicylic acid, an inhibitor of prostaglandin synthesis, on iron absorption in vivo.

*Methods.* Male Wistar rats (200 ± 20 g) were bled by cardiac puncture 1.5 ml per 100 g b.wt during 2 consecutive days. 24 h later another bleeding of 0.75 ml per 100 g b.wt, was performed. After these 3 days of bleeding the animals developed an anemia with hemoglobin values below 10 g%. The rats were then starved during 24 h and forthwith divided in 2 groups: group I (control) receiving $^{59}Fe$ via a catheter placed in the stomach (0.5 μCi or equivalent to 600,000 cpm), together with an iron carrier of ferrous sulphate; group II (experimental) treated in the same way as group I but also receiving 10 mg of acetylsalicylic acid (ASA). The amount of $^{59}Fe$ was monitored in blood, liver,

Effect of acetylsalicylic acid (ASA) on the incorporation into several tissues of orally given $^{59}Fe$

| Variables | Control* (n = 8) | ASA** (n = 9) | Significance*** |
|---|---|---|---|
| Body weight (g) | 195 ± 10 | 194 ± 10 | NS |
| Hemoglobin (g%) | 9.6 ± 0.2 | 9.6 ± 0.4 | NS |
| Hematocrit (%) | 32 ± 1 | 32 ± 1 | NS |
| Blood (cpm in 2 ml/100 g b.wt) | 598 ± 150 | 1733 ± 437 | $p < 0.02$ |
| Spleen (cpm/100 mg w.wt) | 192 ± 48 | 511 ± 130 | $p < 0.02$ |
| Liver (cpm/100 mg w.wt) | 37 ± 3 | 55 ± 8 | $p < 0.02$ |
| Wet washed empty intestine (cpm/100 mg w.wt) | 3051 ± 706 | 2234 ± 625 | NS |
| Freshly removed intestine (cpm/100 mg w.wt) | 278 ± 40 | 311 ± 82 | NS |
| Dry intestine (cpm/100 mg w.wt) | 1379 ± 227 | 1626 ± 479 | NS |
| Femur | 551 ± 119 | 984 ± 170 | $p < 0.05$ |

* Determinations made of 180 min following ingestion (see 'methods' section). ** Means ± SEM. *** Student's t-test.