The action of various volatile anaesthetics on the motility of the rat uterus *in vivo*

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The mechanism of the uterine depressant effects of volatile anaesthetics is not clear. Direct uterine depressant effects have been demonstrated *in vivo* for chloroform in dogs (Van Lierre, Bell & others, 1964) and halothane in women (Embrey, Garrett & Pryer, 1958). Halothane also abolishes uterine responsiveness to oxytocin in women (Miller & Stoetling, 1965). *In vitro* studies have demonstrated a direct uterine depressant action for some volatile anaesthetics including halothane (Munson & Embro, 1977; Naftalin, McKay & others, 1977). The release of catecholamines has been shown to occur with anaesthetics such as ether and chloroform in the cat (Elmes & Jefferson, 1942) and urethane and phenobarbitone sodium in the rat (Spriggs, 1965). Using the rat as an experimental model for recording intra-uterine pressure *in vivo*, the extent to which the uterine depressant action of several volatile anaesthetics involves an indirect component *via* adrenal medullary catecholamine release was investigated using the β-adrenoceptor blocking agent, (±)-propranolol (Black, Duncan & Shanks, 1965) or adrenalectomy or both together.

Timed primiparous pregnant rats (Sprague-Dawley), 250–300 g were housed individually in standard conditions of light (0700–1900 h) and temperature (22°). Day 1 of pregnancy was indicated by spermatozoa in the morning vaginal smear. On the morning of Day 21 of pregnancy the rats were anaesthetized with ether and prepared with an intra-uterine microballoon for recording uterine activity and an indwelling jugular cannula for the injection of drugs, as previously described (Whalley & Riley, 1977). The rats were divided into two groups. One group was adrenalectomized and maintained on 0·9% saline, the other group was sham operated. From Day 22 onwards, the pressure changes within the uterus were recorded continuously using a Bell and Howell pressure transducer connected to a Grass polygraph recorder. Uterine activity was quantified continuously by means of a Grass polygraph integrator. On the first day *post-partum*, oxytocin was infused (1 mU min^{-1}) intravenously by means of a Braun constant rate infusion pump (0·4 ml h^{-1}).

A standard technique for administering each anaesthetic was used. 5 ml of each anaesthetic was placed in a 75 ml beaker containing a wad of cotton wool which was then sealed. After 10 min the lid was removed and the beaker placed over the head of the rat such that the animal lost consciousness within the 1 min exposure period. This standard procedure was repeated after an intravenous injection of the (±)-propranolol (100 μg). The effect of adrenaline (25, 50 and 100 ng) was also investigated. Each rat acted as its own control. Activity of the uterus was compared over a 10 min period for 5 min before and after each procedure in both the sham control group and the adrenalectomized groups.

All the volatile anaesthetics used—diethyl ether, trichloroethylene, halothane and chloroform produced marked reductions of the oxytocin-induced uterine contractions (Table 1). (±)-Propranolol (100 μg, i.v.) had no effect on the oxytocin-induced uterine contractions. Pretreatment with (±)-propranolol (100 μg) reduced the effect of all the volatile anaesthetics. Responses to diethyl ether and trichloroethylene were almost completely abolished but chloroform and halothane still produced marked effects.

The effect of each volatile anaesthetic on the oxytocin-induced uterine activity of the 1-day *post-partum* rat before and after pretreatment with (±)-propranolol is shown in Fig. 1. In the group of 1-day *post-partum* rats which had been adrenalectomized the effect of each volatile anaesthetic was similar to that seen in the (±)-propranolol pretreated sham control rats (Table 1). This small residual uterine depressant effect of each volatile anaesthetic after adrenalectomy was sensitive to pretreatment with (±)-propranolol, except for diethyl ether which produced a mean overall increase in uterine activity.

Adrenaline (25, 50, 100 ng, i.v.) produced a dose-

Table 1. *The effect of 4 volatile anaesthetic agents and adrenaline (25–100 ng, i.v.) on the oxytocin-induced (1 mU min^{-1}, i.v.) uterine contractions of the 1-day* (post-partum) *rat.* Values are given as percentage reduction (mean ± s.e.) in activity. Each animal acted as its own control. Values are shown for intact and adrenalectomized rats before and after treatment with 100 μg (±)-propranolol (n = 3–6 in each group).

	Intact controls		Adrenalectomy	
	Before propranolol	After propranolol	Before propranolol	After propranolol
Agent				
Chloroform	59·2 ± 9·1	17·0 ± 1·9	14·0 ± 2·5	9·4 ± 3·5
Halothane	57·6 ± 15·7	16·6 ± 2·9	16·9 ± 1·1	14·6 ± 2·7
Trilene	61·9 ± 2·8	8·6 ± 2·5	6·1 ± 2·6	5·4 ± 3·1
Ether	36·4 ± 5·5	6·9 ± 1·3	5·4 ± 1·3	8·8 ± 1·9
Adrenaline				
25 ng	38·9 ± 10·1	4·9 ± 0·9		
50 ng	46·8 ± 8·0	7·9 ± 4·7		
100 ng	79·4 ± 9·4	11·9 ± 4·1		

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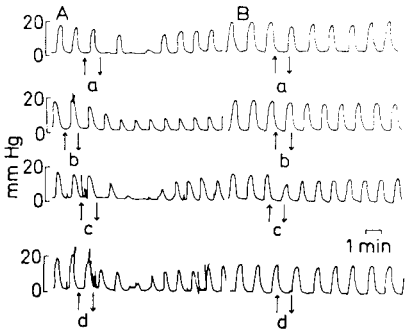

FIG. 1. The effect of a—chloroform, b—diethyl ether, c—halothane and d—trichloroethylene on the oxytocin-induced (1 mU min^{-1}, i.v.) uterine contractions of the 1-day *post-partum* rat (A) before and (B) after pretreatment with (±)-propranolol (100 μg, i.v.).

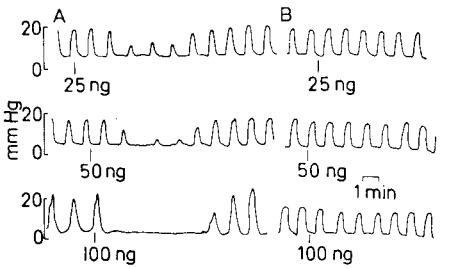

FIG. 2. The effect of 25, 50 and 100 ng adrenaline (i.v.) on the oxytocin-induced (1 mU min^{-1}, i.v.) uterine contractions of the 1-day *post-partum* rat (A) before and (B) after pretreatment with (±)-propranolol (100 μg, i.v.).

dependent reduction of the oxytocin-induced uterine contractions of the 1-day *post-partum* rat which was blocked by pretreating the rats with 100 μg (±)-propranolol (Table 1 and Fig. 2).

The results suggest that diethyl ether, chloroform, trichloroethylene and halothane can depress the oxytocin-induced uterine contractions of the 1-day *post-partum* rat at least in part by an indirect action *via* release of catecholamines from the adrenal medulla. Elmes & Jefferson (1942) have demonstrated in the cat the release of catecholamines from the adrenal medulla by the volatile anaesthetics, ether and chloroform. The catecholamines released would then be available for activation of β-adrenoceptors which are present in the myometrium (Alquist, 1948). Both adrenalectomy and treatment with (±)-propranolol (Black & others, 1965) reduced the uterine-depressant activity of all the volatile anaesthetics. Van Lierre & others (1964), showed that ether and chloroform depressed the activity of the dog uterus and concluded that this was a direct effect of the anaesthetics on the myometrial fibres since there was no uterine depressant action with adrenaline. Our study clearly demonstrates a dose-dependent reduction of oxytocin-induced uterine activity with adrenaline (Table 1) and that this is blocked by a dose of (±)-propranolol known to be effective at the β-adrenoceptor (Barrett & Cullum, 1968). This dose of (±)-propranolol also blocked the uterine depressant action of the volatile anaesthetics.

A direct component may also be involved since pretreatment with (±)-propranolol or adrenalectomy did not completely block the uterine depressant actions of each anaesthetic.

Several studies have shown direct depressant effects of a wide range of volatile anaesthetics, including halothane, particularly on isolated uterine preparations, including pregnant and non-pregnant human uterine muscle (Munson & Embro, 1977; Naftalin & others, 1977) and isolated rat uterus (Anderson & Miller, 1975). The depressant effect of halothane and other volatile anaesthetics on the oxytocin-induced uterine contractions of the rat does not involve direct activation on β-adrenoceptors since propranolol failed to block this effect (Anderson & Miller, 1975). Moreover, propranolol was shown to have antioxytocin activity and also to enhance the uterine depressant action of the volatile anaesthetics. This antioxytocin activity of propranolol on the isolated rat uterus has also been demonstrated by Saini & Sharma (1971) and is a property shared by each of its optical isomers and these authors concluded that the effect was not due to either β-adrenoceptor blockade or local anaesthetic activity. In the present study (±)-propranolol had no antioxytocin activity and blocked the uterine depressant activity of the volatile anaesthetics and adrenaline. Variation in experimental models, doses of oxytocin, halothane and propranolol used may account for the differences in results obtained. In this study we have investigated the effects of a short time period of exposure to a range of volatile anaesthetics which may primarily involve an indirect component depressing uterine activity.

The results presented suggest that several volatile anaesthetics; diethyl ether, chloroform, trichloroethylene and halothane, all depress uterine activity in the rat, which involves an indirect component releasing catecholamines from the adrenal medulla.

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Methadone *N*-oxide in the rhesus monkey

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Conflicting observations have created uncertainty concerning the validity of methadone *N*-oxide as a metabolic product of methadone. Methadone *N*-oxide was first isolated and identified as a metabolite of methadone in hepatic microsomal preparations from several animal species (Beckett, Mitchard & Shihab, 1971). This metabolite has since been isolated from the urine of addicts on methadone maintenance and in patients after a single dose of methadone (Beckett, Vaughan & Essien, 1972). Misra, Mulé & others (1973) and Misra, Bloch & others (1974) found methadone *N*-oxide in the urine of rats and dogs after administration of radiolabelled methadone and suggested that it was a major metabolite of methadone. Other investigators (Baselt & Casarett, 1972; Sullivan & Due, 1973; Änggärd, Gunne & others, 1975) were unable to demonstrate the presence of methadone *N*-oxide as a metabolite of methadone, and one group (Sullivan, Due & McMahon, 1973) suggested that it occurs as an artifact in the urine of man and is probably due to improper storage of urine samples.

In the Rhesus monkey 6 h after intramuscular injection of 0·25 mg kg^{-1} (135 mCi $mmol^{-1}$) of [1-^{3}H]-(—)-methadone HBr (New England Nuclear, Boston, Mass.) a metabolite of methadone was found in all tissues and fluids assayed by t.l.c. (Davis & Fenimore, 1975) which corresponded to the R_F value of a methadone *N*-oxide standard (standard kindly supplied by Prof. A. H. Beckett, London). When [1-^{3}H]-(—)-methadone HBr was added to a tissue homogenate and chromatographed 0·66% ± 0·11 of the radioactivity was assayed to be methadone *N*-oxide.

To minimize the production of methadone *N*-oxide as an artifact of sample preparation, precautions were taken. After dissection, tissues were immediately rinsed with cold physiological saline, blotted dry, sealed in plastic bags and frozen. The frozen samples were crushed in liquid N_2 before homogenization mixed with 4 volumes of water in a cooled glass tube fitted with a Teflon pestle (Arthur Thomas Company, Philadelphia) and homogenized with a variable speed stand mounted motor (Venitron Medical Products, Inc., Carlstadt, N.J.). After extraction with diethyl ether, the organic layer was removed under a stream of N_2 before separation by t.l.c. Because diethyl ether in the presence of air and light produces diethyl peroxides and peroxides form methadone *N*-oxide with methadone, care was taken to avoid using diethyl ether contaminated with peroxides. Newly opened containers of ether were used and peroxide was monitored with potassium iodide-starch test paper.

With these precautions taken, we found a zone of radioactivity appeared on the thin-layer chromatograms which matched the methadone *N*-oxide standard. The percentages of radioactivity in the tissues or fluids which represent methadone *N*-oxide range from insignificant amounts (0·3%) in urine 6 h after drug administration to higher values in spleen (29%), spinal cord (13%), lung (19%), heart (16%), adrenals (22%). To evalulate the possible formation of the *N*-oxide metabolite as an artifact, a kidney homogenate was extracted, dried and redissolved in benzene–methanol (1 : 1 v/v), and periodically for about one and a half months portions of this extract were rechromatographed nine times. The mean percentage of methadone *N*-oxide was 3·46 with a variance of 0·11 when the sample was held at 4° between analysis. When the sample was stored at 30° for 25 days the final analysis showed a twofold increase (7·14%) in the amount of methadone *N*-oxide. Therefore, with adequate safeguards, such as minimizing the oxidative environment, i.e. drying under N_2 and refrigeration, the artifactual formation of methadone *N*-oxide was minimized. While methadone *N*-oxide does occur as an oxidative product of metha-

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