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Influence of Various Anaesthetic Agents on the Formation and Stability of Haemostatic Plugs in the Rabbit Mesentery

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ABSTRACT

The effect of five different anaesthetic agents (urethane, chloralose, pentobarbital, fentanyl-fluanisone and ether) on the formation and stability of haemostatic plugs in transected microvessels of the rabbit mesentery was studied. Ether significantly decreased the frequency of rebleeding through previously stable haemostatic plugs. There were no significant differences between the other four agents although there was a tendency to decreased plug stability during pentobarbital anaesthesia. It is concluded that, despite some theoretical disadvantages, urethane offers the most convenient and satisfactory form of anaesthesia for studies on haemostatic plug formation using the rabbit mesenteric preparation.

INTRODUCTION

Observations on the response to microvascular transection or puncture in the mesentery of anaesthetised animals has been extensively used as a means of studying haemostatic plug formation. Different workers using the mesenteric technique have employed a wide variety of anaesthetic agents. Thus barbiturates (11, 12, 14, 15, 19, 24, 25), chloralose (16, 17), urethane (1, 8, 14, 17), morphine (16) and ether (1) are among the different anaesthetic agents which have been used. The milieu in which platelets aggregate to form a haemostatic plug should appropriately be considered prior to interpretation of results obtained using the method. This view is strengthened by recent reports of anaesthetic-induced changes in platelet reactivity *in vivo* (6, 20). A study of the effect of five commonly used anaesthetic agents on the formation and stability of haemostatic plugs was therefore undertaken to define the suitability of different forms of anaesthesia in studies of the initial haemostatic mechanism, using the rabbit mesenteric preparation.

MATERIALS AND METHODS

Material

New Zealand white rabbits (weight 2.5±0.5 kg), fed on a standard diet (Teknosan pellets, AB Ferrosan, Malmö, Sweden) were used. Five groups, each of 5 aminals, were studied.

1. Ethylcarbamate (Urethane, Kebo, Stockholm, Sweden)
2. Alpha-D-glucochloralose (Chloralose®, Merck, Darmstadt, West Germany)
3. Sodium pentobarbital (Nembutal®, Abbott Laboratories Ltd., North Chicago, USA)
4. Fentanyl+fluanisone (Hypnorm® ad us. vet., AB Leo, Hälsingborg, Sweden)
5. Diethylether (Aether ad narcosin, Skånska Bomullskrutsfabriks AB, Dösjebro, Sweden).

In a further 3 groups, each of 5 animals, the effects of anaesthesia with urethane, pentobarbital or ether on arterial blood pH, pCO_2 and pO_2 were studied.

Anaesthetic technique

1. Urethane was administered i.v. as a 20% solution in 0.9% saline. Supplementary doses were given intermittently as required to a total amount of 1.4±0.1 g/kg.

2. Chloralose anaesthesia was induced using 5% chloralose in 5% sodium borate given i.v. (2) and maintained by intermittent i.v. infusion of 0.8% chloralose in saline. This solution was kept at 30 to 40°C and continuously stirred and before injection it was filtered. Local anaesthesia with 1% xylocaine (Astra Läkemedel AB, Södertälje, Sweden) was needed for skin incision to suppress the hyperexcitability on tactile stimulation after cloralose infusion. The amount of chloralose used for induction was 68.0±16.4 mg/kg and for maintenance 35.3±30.5 mg/kg.

3. Sodium pentobarbital was administered slowly i.v. as a 12.5 mg per ml solution in physiological saline. Small fortification doses were given as required. The total amount used was 36.2±6.9 mg/kg.

4. Neurolept analgesia was achieved by intermittent i.m. injection of Hypnorm (fentanyl 10 mg/ml and fluanisone 0.2 mg/ml). The amount used was 0.82± 0.20 ml/kg.

5. Ether anaesthesia was achieved by drip on open mask.

The injection were given into the marginal ear vein. With each agent, anaesthesia was kept at a depth sufficient to inhibit the blink and paw reflex while maintaining regular respiration. The depth of anaesthesia was assessed every 5 minutes.

Operative technique

The rabbits were starved for about 12 hours before the experiment. Before anaesthesia, a central ear artery was cannulated (Silastic®, Dow Corning, Midland, USA) for sampling and blood pressure measurement before and during induction. After induction, blood pressure was measured through a Teflon cannula (AB Stille-Werner, Stockholm) placed in the femoral artery, using a strain gauge pressure transducer (Statham) and an Ultralette UV recorder (ABEM, Stockholm). Pressure measurements were not obtained during induction with ether. The heart rate was obtained from the pressure curves.

The procedure for haemostatic experiments is described in detail elsewhere (3, 4). Briefly, mesenteric arterioles and venules, each divided according to size into two groups, 20–<40 μm and 40–<60 μm, were cleanly transected, using a fresh Gillette scalpel blade, shape E/11. The primary haemostatic plug formation time, i.e. from transection to the first cessation of bleeding, and the time and frequency of rebleeding through previously stable haemostatic plugs were recorded. The sum of the primary haemostatic plug formation time and the rebleeding times in one vessel gives the total haemostatic plug formation time. In each animal 4 transections in each of the two arteriolar subgroups and 5 transections in each of the two venular subgroups were made. Each transected vessel was observed for 15–20 minutes.

Laboratory technique

Blood from the central ear artery was taken for determination of haematocrit and coagulation time before and 30 minutes after the start of the experiment and at the end of the experiment. Haematocrit was measured in triplicate using a micro-haematocrit centrifuge (10000 g for 5 min; International Equipment Co., Boston, USA). Coagulation time on 1 ml whole blood was determined in duplicate in unsiliconised glass tubes. At the same time intervals the bleeding time after cutting small peripheral ear veins, 0.2–0.5 mm, was measured. The mean value of four bleeding times was calculated on each occasion. In the 15 animals used for acid-base studies blood samples were obtained through a cannula inserted into the central ear artery before and at 15, 30, 60, 90 and 120 minutes after the induction of anaesthesia. The animals were otherwise prepared in the same way as the rabbits used for the haemostatic plug experiments. pH, pCO_2 and pO_2 were measured with an acid-base analyzer (model 213, Instrumentation Laboratory Inc., Lexington, USA).

Statistical methods

The haemostatic plug formation times from transected mesenteric vessels have a skew distribution (4). For statistical purposes the original values were rendered more normally distributed by logarithmic transformation. The mean logarithmic haemostatic plug formation time for each vessel type in each group was calculated and the group means compared by one-way-layout analysis of variance (23). When this test showed significant differences between the groups, simultaneous 95% confidence limits were calculated for differences between the groups according to the T- and S-methods of Scheffé (22). The frequency of rebleeding was calculated for both vessel types and subgroups together for each animal and the differences in the frequencies compared using the rank sum test (9). This test avoids assumptions on the nature of the frequency distribution of the data. The significance of changes in haematocrit, coagulation time, bleeding time, pH, pCO_2, pO_2 and blood pressure was examined by the Student *t*-test.

RESULTS

The depth of anaesthesia was kept at an even level by frequent reassessment of the blink reflex, paw reflex and the rate and depth of respiration. Despite this, two animals in the chloralose group died of respiratory failure. They were excluded from the study. Under cloralose anaesthesia the rabbits tended to be hyperexcitable with excessive reaction to noise and exaggerated tendon reflexes. With neurolept analgesia, there is no real anaesthesia, only profound sedation and analgesia.

The values of pH, pCO_2 and pO_2 in three groups studied are shown in Table I. There was a significant decrease of pH at 15 minutes in the ether group, a slight but significant increase of pO_2 at 90 minutes in the urethane group and a significant decrease of pO_2 at 15 minutes in the pentobarbital group.

During induction the mean arterial pressure increased between 20 and 30 mm Hg in animals anaesthetised with urethane and chloralose, somewhat less after sodium pentobarbital and not at all after neurolept analgesia (Fig. 1). Thereafter, blood pressure in the urethane group decreased and remained constant at the control value while after chloralose, arterial pressure remained approximatively 10 mmHg above the control value. The immediate increase in pressure after sodium pentobarbital injection was followed by a progressive decline throughout the period of observation. After both neurolept analgesia and ether anaesthesia a gradual fall below control values was observed. The changes in arterial blood pressure within and between the different groups were not statistically significant.

The heart rate increased significantly after in-

Table I. *Effect of urethane, sodium pentobarbital and ether on arterial pH, pCO_2 and pO_2*

Mean value and S.D. of 5 experiments in each group
*$= p < 0.01$

	pH	pCO_2 (mmHg)	pO_2 (mmHg)
Urethane			
Control	7.43±0.03	34.1±0.9	77.9±1.5
15 min	7.44±0.05	35.6±1.4	79.1±8.0
30 min	7.44±0.06	35.9±1.1	77.6±4.7
60 min	7.46±0.06	35.4±0.6	80.9±6.7
90 min	7.45±0.08	34.6±1.4	85.0±4.6*
120 min	7.45±0.05	35.1±1.4	81.0±4.1
Sodium pentobarbital			
Control	7.49±0.03	34.4±3.4	80.3±5.7
15 min	7.47±0.05	36.2±1.8	70.2±1.9*
30 min	7.47±0.04	36.8±3.1	71.3±4.6
60 min	7.48±0.04	36.9±3.5	72.0±7.1
90 min	7.51±0.05	35.9±2.8	78.9±7.6
120 min	7.48±0.05	36.9±2.1	76.0±4.4
Ether			
Control	7.43±0.03	33.1±3.1	87.2±4.7
15 min	7.31±0.07*	31.3±2.4	91.9±9.3
30 min	7.37±0.08	29.8±2.9	90.7±8.8
60 min	7.39±0.06	29.5±6.2	89.8±13.2
90 min	7.38±0.07	30.0±5.0	86.6±9.8
120 min	7.36±0.08	32.2±4.4	85.9±12.0

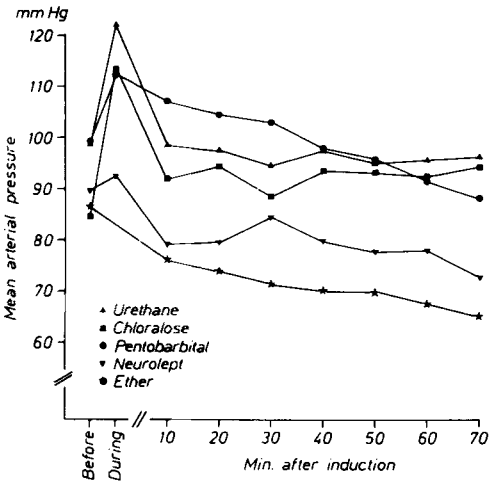

Fig. 1. The effect of different anaesthetic agents on mean arterial blood pressure before and during induction of anaesthesia and at intervals after start of experiment. Each point represents the mean of 5 observations.

duction and remained elevated in all groups ($p < 0.005$) except neurolept analgesia where no change occurered.

Changes in the bleeding time from small ear veins and in coagulation time and haematocrit are shown in Table II. The haematocrit remained about the same level throughout the experiment in all the groups. The coagulation time showed a tendency to increase, but this change was not statistically significant in any group. Variable fluctuations in bleeding time were seen in the different groups. The only value of statistical significance was the decrease in bleeding time at 30 minutes after sodium pentobarbital anaesthesia ($p<0.01$) and this was followed by a return to near control values by the end of the experiment.

The total and primary haemostatic plug formation times from transected mesenteric vessels is shown in Table III. Analysis of variance revealed no significant differences between the groups. The frequency of rebleeding (Table IV) was significantly less for arterioles than for venules in the different groups ($p<0.005$) except for ether. Table IV also shows that the venules in the ether

Table II. *Effect of different anaesthetic agents on bleeding time (BT, seconds), coagulation time (CT, seconds) and haematocrit (Hct,%) before induction of anaesthesia and at 30 and 70 minutes after start of experiment*

Mean values and S.D. of 5 experiments in each group
*$= p < 0.01$

	Control	30 min.	70 min.
Urethane			
BT	76±11	53±14	51±14
CT	259±87	288±47	315±42
Hct	38.8±2.6	40.2±3.5	37.8±1.9
Chloralose			
BT	58±9	75±10	43±9
CT	291±20	303±34	330±32
Hct	37.8±1.9	36.4±4.2	37.0±4.1
Sodium pentobarbital			
BT	62±11	41±5*	55±6
CT	228±34	249±58	267±37
Hct	39.0±3.4	39.4±2.8	39.6±3.0
Neurolept			
BT	66±7	72±21	51±11
CT	225±30	270±28	263±29
Hct	36.4±1.1	34.4±1.5	35.8±2.2
Ether			
3T	68±24	64±13	62±18
CT	243±63	273±34	270±28
Hct	34.2±4.1	35.2±3.6	33.0±5.1

Table III. *Effect of different anaesthetic agents on total (THT) and primary (PHT) haemostatic plug formation times (seconds) in transected mesenteric arterioles and venules*

Each value represents the mean and S.D. of 20 observations on transected arterioles and 25 observations on transected venules in each vessel subgroup

	Arteriole		Venule	
	20–<40 μm	40–<60 μm	20–<40 μm	40–<60 μm
Urethane				
THT	31±11	84±32	280±135	298±143
PHT	25±8	76±31	177±132	170±137
Chloralose				
THT	89±88	138±90	259±104	273±131
PHT	87±90	101±39	179±110	168±89
Sodium pentobarbital				
THT	68±13	54±33	354±146	389±190
PHT	59±13	47±33	144±49	243±183
Neurolept				
THT	136±114	102±62	187±174	393±151
PHT	132±115	97±62	132±124	257±150
Ether				
THT	90±86	79±46	138±59	328±217
PHT	77±86	69±44	116±74	267±225

group had a significantly lower rebleeding frequency than venules in the other four groups ($p<0.05$).

In Fig. 2 the total frequency of rebleeding has been plotted against the total rebleeding time. It is apparent that, particularly in venules, sodium pentobarbital and ether differed from the other three anaesthetic agents used. The pentobarbital group showed a tendency to a long total rebleeding time while the opposite is true of ether. There were, however, no statistically significant differences between the total rebleeding times in the different groups.

Table IV. *Incidence of rebleeding through previously stable haemostatic plugs in different anaesthetic groups*

The low incidence of rebleeding after ether anaesthesia is apparent while the values for the other groups are comparable

	Urethane	Chloralose	Sodium pentobarbital	Neurolept	Ether
Arteriole, 20–<40 μm	6	3	6	5	2
Arteriole, 40–<60 μm	4	8	6	3	3
Venule, 20–<40 μm	18	12	19	14	3
Venule, 40–<60 μm	20	16	18	18	7
Total	48	39	49	40	15

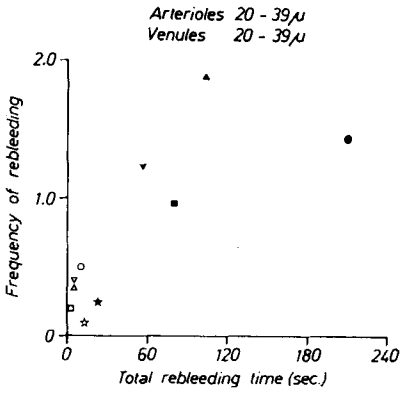

Fig. 2. Total rebleeding time in transected mesenteric arterioles (open symbols) and venules (filled symbols) plotted as a function of the frequency of rebleeding. The symbols representing each anaestetic group are as shown in Fig. 1.

DISCUSSION

It has become increasingly accepted by workers studying, in particular, the cardiovascular system that anaesthesia has a profound effect on differrent physiological functions. When interpreting data obtained from anaesthetised animals it is clearly important to estimate the extent of possible abnormality.

In this study, ether was the only anaesthetic agent which significantly altered haemostatis; the haemostatic plug formation times from transected mesenteric venules were somewhat shorter and the incidence of rebleeding through haemostatic plugs in venules was significantly lower. Ether also induced most hypotension of the various anaesthetics used. Since, in addition to these disadvantages, assistance is required to maintain an even depth of ether anaesthesia, use of this agent appears unsatisfactory in these experiments. The technical difficulties associated with the use of neurolept or chloralose anaesthesia were not offset by any obvious advantage with either agent.

The relatively stable acid-base status during urethane anaesthesia in rabbits has been reported by Bito & Eakins (5) and emphasis had also been placed on the wider safety margin of urethane

over barbiturate anaesthesia (21). From a circulatory point of view urethane seems to give more stable conditions (18). pH, pCO_2 and pO_2 were stable during urethane anaesthesia, thus confirming the results of Bito & Eakins (5). The significant reduction in pO_2 immediately after induction of pentobarbital anaesthesia confirms our earlier results (20).

Although both urethane and pentobarbital have on occasion been given intraperitoneally in haemostatic and thrombotic studies (8, 12, 24), this route seems questionable when observations on the mesenteric microvasculature are being made.

The present study shows that the haemostatic plug formed during pentobarbital anaesthesia tends to be unstable, this tendency being reflected as a longer total rebleeding time. Born & Philp (6) showed that the duration of platelet micro-embolisation from sites of injury in rat pial vessels was significantly longer after urethane anaesthesia than after pentobarbital or ether. Since control observations could not be made on unanaesthetised rats it is difficult to draw precise conclusions, particularly since an inhibitory effect of pentobarbital on rabbit platelet reactivity *in vitro* and *in vivo* has recently been reported (20).

The cytotoxic effect of urethane on vascular endothelium (10) and its haemolytic effect (7) are the theoretical objections to acceptance of it as the anaesthetic of choice for these studies. In the concentration used (20% w/v) urethane causes minimal haemolysis and any adenosine diphosphate released from red blood cells would be rapidly metabolised (13). Since the ideal anaesthetic agent in these experiments should lie between the extremes of negligible rebleeding (if situations of decreased plug stability are under study) and a pronounced rebleeding tendency (if situations of enhanced plug stability are being examined), the compromise which fits best these circumstances would appear to be urethane.

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