Laboratory Investigation

Temperature-dependent effects of halothane and isoflurane on the isolated left atrium

M.L. Laorden,‡ F.S. Miralles,* M.D. Cárceles,* J. Hernández,‡ M.M. Puig,†

The aim of the present study was to examine whether changes in temperature alter the effects of halothane and isoflurane on isolated left atria. Concentration–response curves for inotropic effects at different temperatures (30° C, 37° C, 40° C) on electrically stimulated left atria of the rat were obtained. The change of temperature modified the maximal negative inotropic response to halothane. The maximal decrease induced by halothane was 12 ± 2.3 per cent at 37° C and 18 ± 2.5 per cent at 30° C. When the temperature increased up to 40° C the maximal decrease of atrial inotropism was 46 ± 2.1 per cent – significantly higher than obtained at 37° C. However, the maximal effect obtained by isoflurane was not significantly affected by temperature (30° C = 7 ± 1.6 per cent; 37° C = 8 ± 1.8 per cent; 40° C = 2 ± 0.8 per cent). Furthermore the potency of halothane (expressed as the concentration which produced 50 per cent inhibition – IC 50 per cent), decreased significantly at 30° C (IC 50 = 1.34 ± 0.18) and increased at 40° C (IC 50 = 0.44 ± 0.17) when compared with its potency at 37° C (IC 50 = 0.96 ± 0.08). On the other hand changes in temperature did not significantly modify the IC 50 for isoflurane obtained at 37° C. These data demonstrated that the maximal effects of halothane but not those of isoflurane on the left atria preparation were modified by change in temperature; the potency of halothane but not of isoflurane increased significantly with increases in temperature of the organ bath.

Le but de la présente étude était d'examiner si les changements de la température altèrent les effets de l'halothane et de l'isoflurane sur l'oreillette gauche isolée. Les courbes de concentration-réponse pour les effets inotropes à différentes températures (30° C, 37° C, 40° C) sur l'oreillette gauche de rat stimulé électriquement furent obtenues. Le changement de température a modifié la réponse inotropique négative maximale à l'halothane. La diminution maximale induite par l'halothane était de 12 ± 2,3 pour cent à 37° C et 18 ± 2,5 pour cent à 30° C. Quand la température augmenta jusqu'à 40° C la diminution maximale de l'inotropisme de l'oreillette était de 46 ± 2,1 pour cent significativement supérieure que celui obtenu à 37° C. Cependant l'effet maximal obtenu par l'isoflurane n'était pas significativement affecté par la température (30° C = 7 ± 1,6 pour cent; 37° C ± 1,8 pour cent; 40° C = 2 ± 0,8 pour cent). De plus la puissance de l'halothane (exprimé comme étant la concentration qui produit 50 pour cent d'inhibition – IC 50 pour cent), diminua significativement à 30° C (IC 50 = 1,34 ± 0,18) et augmenta à 40° C (IC 50 = 0,44 ± 0,17) lorsque comparée à sa puissance à 37° C (IC 50 = 0,96 ± 0,08). D'autre part les changements de température n'ont pas modifié significativement le IC 50 pour l'isoflurane obtenu à 37° C. Ces donnés démontrent que les effets de l'halothane et non ceux de l'isoflurane sur la préparation d'oreillette étaient modifiés par les changements de température; la puissance de l'halothane et non celle de l'isoflurane augmenta significativement avec l'augmentation de la température.

Key words

ANAESTHETICS, VOLATILE: halothane, isoflurane;
HEART: atria, contractility, inotropism;
HYPOTHERMIA;
HYPERTHERMIA.

From the Departments of *Anesthesiology, Virgen Arrixaca Hospital, Murcia, Spain, and †New York University Medical Center, New York, and ‡Department of Physiology and Pharmacology, Medical School, Murcia, Spain.

Address correspondence to: Dr. F.S. Miralles, Servicio Anestesiologia, Hospital Virgen de la Arrixaca, Murcia, Spain.

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The consequences of changes in temperature on the activity and dose requirements of volatile anaesthetics are poorly defined. However, it is known that the minimal alveolar concentration (MAC) of volatile anaesthetics (as a measure of anaesthetic requirement) increases during hyperthermia[1] and decreases with hypothermia.[2] It is known that hypothermia decreased metabolic heat pro-

duction, increased heat loss to the environment from cutaneous vasodilatation, surgical exposure, and dry respiratory gases and reduced compensatory responses (vasoconstriction, shivering and nonshivering thermogenesis) due to hypothalamic suppression and the effects of muscle relaxants.[3] Moreover, *in vitro* studies have shown that halothane induces calcium-dependent contractures in the rat soleus muscle between 4° C and 13° C, but not at higher temperatures.[4]

The aim of the present study was to examine whether changes in temperature alter the effects of halothane and isoflurane on isolated left atria.

Methods

The experiments were carried out using the isolated left atria preparation. Male Sprague-Dawley rats of either sex and weighing 150–200 g were stunned by a blow to the head and immediately decapitated. The chest was opened by a midsternal incision and the left atrium was isolated by a technique similar to that described by Furchgott.[5] A 30-ml Allihn tube was used as the organ bath, the porous base plate had pore diameters between 3 and 5 μm, to allow effective aeration. Tyrode solution of the following composition (mM) was used: NaCl 163.9; KCl 5.0; $MgCl_2$ 1.05; $CaCl_2$ 1.8; NaH_2PO_4 0.4; $NaHCO_3$ 11.9; dextrose 5.0. The bathing solution was maintained at 30° C, 37° C and 40° C respectively, and bubbled with 95 per cent O_2 and 5 per cent CO_2. The left atrium was placed under a resting tension of 0.5 g and was electrically stimulated by a Grass SD-9 stimulator by means of two platinum ring electrodes with rectangular pulses at a frequency of 0.2 Hz, duration of 5 msec and supramaximal voltage (30 V) for 30 min before starting the experiment. The force of contraction of the left atrium was measured using a force displacement transducer (Grass FT 03) and was recorded on a Beckman Dynograph polygraph. After a 30-min stabilization period cumulative concentration response curves were established. Each drug concentration was added to the organ bath at three-minute intervals. Control experiments without drugs at 30° C, 37° C and 40° C were performed and there were no changes in the force of contraction during the experiments.

The drugs used in this study were halothane (ICI) and isoflurane (Abbott). When the volatile anaesthetics were used the O_2/CO_2 gas mixture was delivered through BOC vaporizers, and the gas flow was adjusted to deliver a given concentration with an Engström Emma multigas analyzer. The range of concentrations tested was from 0.1 to 2.5 v/v per cent. A drug effect was defined as the maximal change in the force of contraction recorded in the two minutes following addition of each drug to the bathing solution.

The results are expressed as percentages relative to the control values of force of contraction of the preparation.

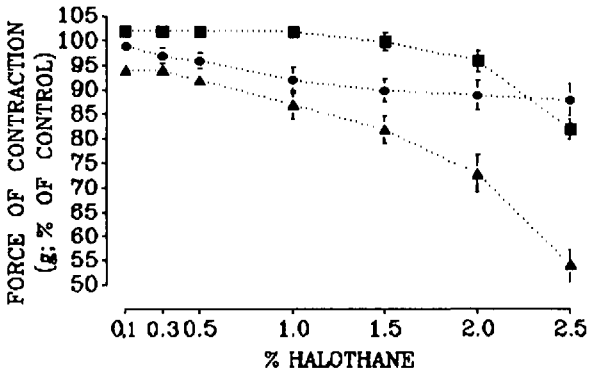

FIGURE 1 Effect of halothane at different temperatures: 30° C (■---■, $n = 5$), 37° C (●---●, $n = 8$) and 40° C (▲---▲, $n = 5$) on the atrial inotropism.

Log dose–response curves were used to determine the inhibitory concentration 50 (IC 50) (the concentration of volatile anaesthetics which produce half of the maximal inhibition of the force of contraction) at 30° C, 37° C and 40° C. Statistical analysis was performed by a two-way analysis of variance (ANOVA) for intergroup comparisons between doses and drugs, with Student's t test for individual comparison. An analysis of linear regression with the log of response for the estimation of IC 50 was also used. A *P*-value less than 0.05 was considered to indicate statistical significance.

Results

Effects of halothane on contractile force

Concentration–response curves for halothane obtained at different temperatures are illustrated in Figure 1. At 37° C all tested concentrations of halothane, except 0.1 v/v per cent, induced significant ($P < 0.01$) decreases in the atrial force of contraction when compared with control. At 30° C atrial force of contraction did not change at halothane concentrations ranging from 0.1–2 v/v per cent, but at the highest concentration tested (2.5 per cent) a small (18 ± 2.5 per cent) but significant ($P < 0.01$) decrease was observed. At 40° C the force of contraction was depressed significantly from control values at 0.5, 1, 1.5, 2, and 2.5 per cent halothane.

The change of temperature modified the maximal negative inotropic response to halothane. The maximal decrease was 12 ± 2.3 per cent at 37° C, 18 ± 2.5 per cent at 30° C, and 46 ± 2.1 per cent at 40° C ($P < 0.001$) (Table I).

Effects of isoflurane on contractile force

Similar concentration–response curves were constructed for isoflurane. At 37° C the atrial force of contraction was not changed with isoflurane at low concentrations (0.1–

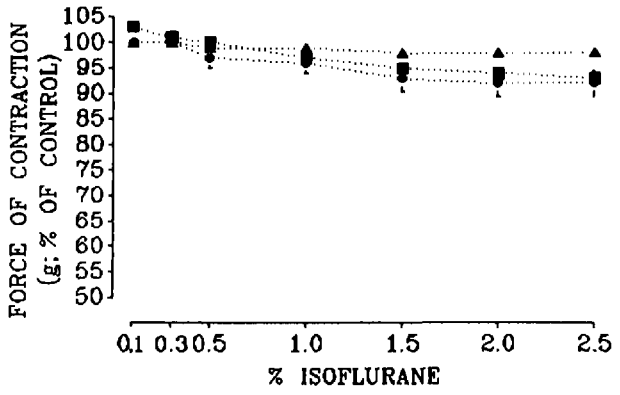

FIGURE 2 Effect of isoflurane at different temperatures: 30° C (■---■, $n = 5$), 37° C (●---●, $n = 6$) and 40° C (▲---▲, $n = 5$) on the atrial inotropism.

0.3 v/v per cent) but a small (8 ± 1.8 per cent maximum) but sigificant ($P < 0.05$) decrease was seen at 2 per cent (Figure 2).

The maximal effect obtained by isoflurane was not significantly affected by temperature (30° C = 7 ± 1.6 per cent; 37° C = 8 ± 1.8 per cent; 40° C = 2 ± 0.8 per cent) (Table I).

The concentrations of halothane that caused a 50 per cent decrease in atrial inotropism (IC 50) at different temperatures are summarized in Table II. At 37° C the IC 50 for halothane was 0.96 ± 0.08. Studies performed at 30° C and 40° C demonstrated a statistically significant change in the potency of halothane. At 30° C the IC 50 for halothane was 1.34 ± 0.18 – significantly higher than

TABLE I Effects of the maximal inotropic response (E Max) to halothane and isoflurane at different temperatures

	Halothane			*Isoflurane*		
T(°C)	*E Max*	*Change*	*P*	*E Max*	*Change*	*P*
37	12 ± 2.3	—	—	8 ± 1.8	—	—
30	18 ± 2.5	6	NS	7 ± 1.6	1	NS
40	46 ± 2.1	34	0.01	2 ± 0.8	6	NS

$n = 5$ for each group except for halothane ($n = 8$) and isoflurane; ($n = 6$) at 37° C.

TABLE II Effects of temperature on the potency of halothane tested upon isolated left atria

	Temperature (°C)		
	30 (n = 8)	*37 (n = 5)*	*40 (n = 5)*
IC 50	1.34 ± 0.18	0.96 ± 0.08	0.44 ± 0.17
Relative potency	0.71	1	2.1

*Significantly different from response at 37° C; $P < 0.05$.

obtained at 37° C. However, at 40° C halothane was twice as potent as the control value (37° C). On the other hand changes in temperature did not significantly modify the IC 50 for isoflurane obtained at 37° C.

Discussion

This study demonstrated that the effects of halothane but not those isoflurane on the left atria preparation were temperature-dependent. The potency of halothane increased significantly with increases in temperature of the organ bath. The range of temperature in this study (30° C, 37° C and 40° C) was chosen because it included the range of temperature experienced by patients during surgery or in the immediate postoperative period.

The effects of temperature on dose requirements of drugs are the result of changes in both pharmacokinetic and pharmacodynamic variables. That is, temperature may change the distribution, biotransformation or excretion of drugs, or the interaction with their ultimate sites of action. We have studied the effects of hypo- and hyperthermia on the potency of halothane and isoflurane on the isolated left atria. The isolated left atria is a classical method which is widely used to assess the inotropic effects for drugs.[6,7] In the present *in vitro* model the negative inotropic effects induced by halothane but not by isoflurane were sensitive to changes of the temperature in the medium. Our results demonstrated that larger doses of halothane were needed to produce a given effect during hypothermia, while lower doses were needed during hyperthermia. In contrast, several studies have demonstrated that the MAC of volatile anaesthetics is reduced during hypothermia.[8,9] It is reduced in a different degree related to the anaesthetic liposolubility.[2] When the temperature decrease from 37° C to 27° C the MAC for halothane, isolflurane and methoxyflurane was reduced about 50 per cent.[2] On the other hand there is a positive correlation between temperature (ranging from 37.3° C to 40.7° C) and the MAC of halothane. The MAC increases by about eight per cent for each centigrade degree increment of temperature. However, when temperature increases above 42° C the value of MAC not only does not increase but is reduced.[1] The discrepancies between these two results could be related to the different models used in each study; *in vitro* experiments are devoid of compensatory mechanisms and pharmacokinetic effects which are present in *in vivo* models. These mechanisms would influence the direct effect of a given drug. The temperature-dependent change in the activity of volatile anaesthetic also could have been caused by several events at the effector site. Pharmacokinetic changes can be ruled out since this *in vitro* preparation does not metabolize or redistribute the volatile anaesthetics. However, variations in temperature could have altered the binding character-

istics of receptors. Although no specific binding sites or receptors have been described for volatile anaesthetics, recently we have demonstrated a synergistic interaction between halothane and morphine in the guinea pig myenteric plexus longitudinal muscle preparation, suggesting that the potentiation is related to events occurring at the opioid receptor complex.[10] It is possible that hyperthermia may also differentially alter the affinity of opioid receptors for agonists and antagonists.[11] Thus, we demonstrated in a previous study that higher concentrations of morphine are needed at the opioid receptor during hypothermia.[12] Therefore we need to take into account the possibility that our higher experimental temperature (40° C) could induce changes in opioid receptor affinity and the possibility that the negative inotropic effect induced by halothane may be mediated, at least in part, by the opioid receptor complex. In this way we have previously demonstrated that naloxone (opioid antagonist) antagonized the inotropic negative effects induced by halothane.[13]

Another possible mechanism whereby volatile anaesthetics produce a negative inotropic effect in normal cardiac muscle is related to alterations in calcium ion flux.[14] The maximal effect obtained with isoflurane was not significantly affected by increases of the temperature. The difference between isoflurane and halothane could be explained according to different action mechanisms. Halothane appeared to have calcium channel blocking activity[15] while isoflurane appeared to affect intracellular calcium kinetics more.[16]

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