Thiopentone inhibits beta-adrenergic responses in myocardial tissue

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The purpose of this study was to determine the importance of inhibition of beta-adrenergic function in thiopentone-induced myocardial depression. Using an isolated, electrically stimulated rat left atria model, contractile dose-response curves to thiopentone (200 μ M, 400 μ M, 600 μ M, 800 μ M) were shifted to the right in preparations treated with 10^{-3} M dibutyryl cyclic adenosine monophosphate (cAMP) compared with atria stimulated with 10^{-6} M isoprenaline, demonstrating that inhibition of beta-adrenergic mechanisms by thiopentone is physiologically important. Depression by thiopentone was similar in atria treated with 10^{-5} M forskolin compared with preparations stimulated with 10^{-6} M isoprenaline, indicating that thiopentone does not block beta-adrenergic receptors. It is concluded that thiopentone depresses myocardial function by several mechanisms, one of which involves inhibition of the adenylyl cyclase cascade. The adenylyl cyclase enzyme is a likely site where thiopentone inhibits the system; however, other components of the cascade may also be involved.

L'objectif de cette étude consiste à déterminer l'influence de l'inhibition de l'activité β -adrénergique sur la dépression myocardique induite par le thiopentone. A l'aide d'un modèle constitué d'une oreillette gauche de rat stimulée électriquement, la relation dose-effet du thiopentone sur la contractilité (200 μ M, 400 μ M, 600 μ M, 800 μ M) se déplace vers la droite dans des préparations traitées avec de l'adénosine monophosphorique cyclique (cAMP) 10^{-3} M comparativement à des oreillettes stimulées avec de l'isoprénaline 10^{-6} M, ce qui démontre que

Key words

ANAESTHETICS, INTRAVENOUS: thiopentone;

HEART: contractility;

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l'inhibition β -adrénergique provoquée par le thiopentone est physiologiquement importante. La dépression de l'oreillette provoquée par le thiopentone est identique à celle que produit la forskoline 10^{-5} M comparativement à celle de l'isoprénaline 10^{-6} M, ce qui indique que le thiopentone n'inhibe pas les récepteurs β -adrénergiques. Les auteurs concluent que le thiopentone déprime la fonction myocardique par plusieurs mécanismes qui impliquent l'inhibition de la cascade de l'adenylyl cyclase. L'inhibition du système se produit vraisemblablement au niveau de l'enzyme adenylyl cyclase; cependant, il est possible que d'autres éléments de la cascade de l'adenylyl cyclase soient impliqués.

Thiopentone is short-acting barbiturate commonly used to induce general anaesthesia. Undesirable depression of cardiovascular function frequently occurs with the use of this agent. Barbiturates indirectly depress cardiovascular homeostasis by inhibiting central nervous sympathetic outflow and act directly on myocardial tissue by blocking voltage-gated calcium channels.¹⁻⁴ Other possible sites where thiopentone may act to decrease myocardial contractility include sarcolemmal calcium binding sites⁵ and sarcoplasmic reticulum.⁶

Beta₁ adrenergic agonists activate myocardial adenylyl cyclase, causing a well-described positive inotropic effect.⁷ Beta₁ adrenergic responses are mediated via external cellular receptors. Attachment of a beta₁ adrenergic agonist to its receptor activates the G protein, G_s, that then combines with adenylyl cyclase and increases the activity of the enzyme. In a similar manner, adenosine and acetylcholine bind to cell receptors and activate G_i that combines with adenylyl cyclase and decreases the enzyme's activity. The function of the myocardial adenylyl cyclase system can also be modified by other agents. Forskolin directly interacts with adenylyl cyclase to increase the activity of the enzyme. Dibutyryl cyclic adenosine monophosphate (cAMP) is a nonhydrolyzable analogue of cAMP that can cross cell membranes and mediate a positive inotropic response that is unaffected by agents that inhibit adenylyl cyclase activity.⁸

The activity of adenylyl cyclase from heart, lung, and

brain membrane preparations is inhibited by barbiturates.⁹⁻¹² Whether barbiturates inhibit the function of the adenylyl cyclase cascade in intact cells has not been extensively studied. Massart *et al.* showed that the TSH-stimulated cAMP levels in isolated thyrocytes are reduced by thiopentone, and this phenomenon is associated with decreased production of thyroglobulin and tri-iodothyroxine.¹³ Vasopressin-induced cAMP production in toad urinary bladder and water flow across the bladder membrane are likewise inhibited by phenobarbitone and methohexitone.¹⁴

Since barbiturates interfere with adenylyl cyclase activity in a variety of tissues including myocardial membrane preparations, we investigated the physiological importance of inhibition of the adenylyl cyclase cascade in contributing to the myocardial depression by thiopentone.

Methods

Determination of thiopentone dose-response curves

After approval by the University of Texas Medical Branch Animal Care and Use Committee, 200–250 g Wistar rats were sacrificed under halothane anaesthesia followed by quick removal of the heart. The left atrium was excised and placed in warmed (37°C) Krebs's solution (composition in millimolar: NaCl 118; KCl 4.6; MgSO₄ 4.9; CaCl₂ 2.5; glucose 11; NaH₂PO₄ 1.2; NaHCO₃ 25) bubbled with 95% O₂, 5% CO₂. The muscles were mounted to a force transducer (Grass, model FT03, Quincy, MA, USA) and 1.0 g base tension was applied. Contractions were induced with an electric stimulator (Grass model S88) at 1.0 Hz (1.0 ms squarewave bursts of 5.0 V) and peak tension was displayed and measured on a polygraph (Gould Electronics, model 2400, Cleveland, OH, USA). The atria were allowed to stabilize for 60 min before experiments began. Baseline contractions remained stable throughout the experimental period.

External field stimulation of isolated cardiac muscle has been shown to cause release of neurotransmitters from peripheral nerve terminals.¹⁵ Because barbiturates can inhibit release of neurotransmitters from peripheral autonomic nerves,¹⁶ maximal doses of atropine (10⁻⁶ M) were added to the bath to avoid confounding the results with this effect. For the same reason, maximal doses of isoprenaline (10⁻⁶ M) were used. Preparations initially were exposed to atropine (10⁻⁶ M) and allowed to stabilize. Isoprenaline (10⁻⁶ M) or dibutyryl-cAMP (10⁻³ M) was then added in the continued presence of atropine. After the muscles had re-equilibrated, dose-response curves were obtained with cumulative doses of thiopentone. Thiopentone was then washed, with all preparations recovering to within 90% of the tension before adding the barbiturate. Similar dose-response curves were ob-

tained using maximal doses of forskolin (10⁻⁵ M). Only one drug was tested on each preparation.

Statistics

Results are expressed as means $\pm$ SEM; *P* values were determined using two-tailed Student's *t* test, with *P* < 0.05 considered statistically significant.

Drugs

Thiopentone was obtained from Abbott Laboratories (North Chicago, IL). All other chemicals and reagents were obtained from Sigma (St. Louis, MO).

Results

Addition of atropine (10⁻⁶ M) to isolated contracting atria caused a small positive inotropic response (peak tension increased to 115 $\pm$ 3.5% of control values, *P* < 0.05). The addition of maximal doses of isoprenaline (10⁻⁶ M) to the bath increased contractile tension to 303 $\pm$ 14% of initial tension, which was not different from the increase in tension by maximal stimulation with 10⁻³ M dibutyryl cAMP (tension increased to 295 $\pm$ 11% of initial tension). The dose-response curve (Figure 1) for thiopentone was shifted to the right in preparations treated with dibutyryl cAMP.

The increase in twitch tension by 10⁻⁵ M forskolin (337 $\pm$ 41% of initial tension) was not different from stimulation with isoprenaline. The depression by thiopentone in these preparations was not different from that observed in isoprenaline-stimulated atria (Figure 2).

Discussion

The results of this study demonstrate that myocardial depression by thiopentone is attenuated in atria stimulated with dibutyryl cAMP when compared to preparations treated with the beta-adrenergic agonist isoprenaline. The myocardial depression induced by thiopentone in forskolin-treated muscle was not different from the depression in atria stimulated with isoprenaline.

Previous work has shown that the activity of adenylyl cyclase from membrane preparations of various tissues can be inhibited by barbiturates.⁹⁻¹² Only two studies have examined the influence of barbiturates on myocardial adenylyl cyclase. Weinryb *et al.*⁹ found that the activity of adenylyl cyclase from guinea pig ventricular homogenates was decreased in the presence of brominated barbiturates, and Khatter *et al.*¹⁰ showed that high doses of pentobarbitone inhibit adenylyl cyclase activity of rat sarcolemmal preparations. An important limitation of these two studies is that inhibition of adenylyl cyclase activity by barbiturates in tissue homogenates may not have a physiological consequence in intact muscle. We performed this study using intact muscle to clarify the im-

FIGURE 1 Dose-response curves to thiopentone in the presence of maximal concentrations of isoprenaline (ISO, 10^{-6} M) or dibutyryl-cAMP (D-cAMP, 10^{-3} M). All experiments were performed in the presence of atropine (10^{-6} M). Data are expressed as a percentage of the contractile tension before thiopentone was administered. * $P < 0.05$ between ISO and D-cAMP groups.

FIGURE 2 Dose-response curves to thiopentone in the presence of maximal concentrations of isoprenaline (ISO, 10^{-6} M) or forskolin (FORS, 10^{-5} M). All experiments were performed in the presence of atropine (10^{-6} M). Data are expressed as a percentage of the contractile tension before thiopentone was administered. There was no difference in the dose-response curves.

portance of inhibition of the adenylyl cyclase cascade in contributing to myocardial depression by thiopentone.

The right-ward shift in the thiopentone dose-response curve in preparations treated with dibutyryl-cAMP compared with isoprenaline-stimulated atria indicates that inhibition of the adenylyl cyclase cascade by thiopentone has physiological importance in cardiac muscle. These results are supported by other studies that have shown that barbiturates inhibit adenylyl cyclase mediated responses in intact cells.^{13,14} However, most of the depression in con-

tractility by thiopentone is not related to alterations of adenylyl cyclase function.

Thiopentone could potentially inhibit the beta-adrenergic system at multiple sites: (1) direct blockade of beta-adrenergic receptors, (2) interference with the coupling of beta-adrenergic receptors to adenylyl cyclase either by alteration of membrane mechanics or G protein function, (3) interference with the activation of adenylyl cyclase by G_s or other activators, (4) direct inhibition of adenylyl cyclase activity, (5) inhibition of pathways activated by adenylyl cyclase, or (6) acceleration of the degradation of cAMP. Forskolin causes a positive inotropic effect by directly activating adenylyl cyclase. Since the dose-response curves to thiopentone were identical in forskolin-treated and isoprenaline-stimulated preparations, direct blockade of beta-adrenergic receptors is an unlikely mechanism that thiopentone decreases contractility. Barbiturates have been shown to directly inhibit adenylyl cyclase activity in rat brain, probably by interfering with the activation of adenylyl cyclase by the G protein, N_s .¹¹ Direct inhibition of myocardial adenylyl cyclase or interference with the activation of adenylyl cyclase by G_s or forskolin are possible mechanisms that thiopentone could attenuate myocardial beta-adrenergic responses. Alternatively, accelerated degradation of cAMP or inhibition of pathways activated by cAMP may also contribute to the reduction of myocardial contractility by thiopentone. It is not possible to determine the exact site or sites that thiopentone inhibits the adenylyl cyclase cascade from the results of this study; however, beta-adrenergic receptor blockade is an unlikely mechanism.

Since we performed this work in isolated tissue, inhibition of adenylyl cyclase activity by thiopentone may have greater or lesser importance in whole animals and human patients. Clinical experience has shown that patients who are highly dependent on sympathetic tone (and therefore adenylyl cyclase activity) to maintain cardiovascular stability tend to have exaggerated depressive responses to thiopentone. It is possible that inhibition of adenylyl cyclase activity may be an important factor in mediating the cardiovascular collapse that is seen with administration of thiopentone in such patients.

In conclusion, our results demonstrate that part of the myocardial depression induced by thiopentone is secondary to inhibition of the adenylyl cyclase cascade. However, other mechanisms are also involved in mediating thiopentone negative inotropy. Inhibition of adenylyl cyclase may be an important mechanism that thiopentone causes an exaggerated cardiovascular depression in patients with high sympathetic tone.

References

- Ikemoto Y. Reduction by thiopental of the slow-channel-

- mediated action potential of canine papillary muscle. *Pflügers Arch* 1977; 372: 285-6.
- 2 Ikemoto Y, Yatani A, Arimura H, Yoshitake J. Reduction of the slow inward current of isolated rat ventricular cells by thiamylal and halothane. *Acta Anaesthesiol Scand* 1985; 29: 583-6.
 - 3 Gilat E, Rubinstein I, Binah O. Effect of sodium pentobarbital on the transmembrane action potential and the slow inward current of guinea pig ventricular myocytes. *J Cardiovasc Pharmacol* 1987; 10: 485-8.
 - 4 Ebert TJ, Kanitz DD, Kampine JP. Inhibition of sympathetic neural outflow during thiopental anesthesia in humans. *Anesth Analg* 1990; 71: 319-26.
 - 5 Nayler WG, Szeto J. Effect of sodium pentobarbital on calcium in mammalian heart muscle. *Am J Physiol* 1972; 222: 339-44.
 - 6 Lain RF, Hess ML, Gertz EW, Briggs FN. Calcium uptake activity of canine myocardial sarcoplasmic reticulum in the presence of anesthetic agents. *Circ Res* 1968; 23: 597-604.
 - 7 Drummond GI, Severson DL. Cyclic nucleotides and cardiac function. *Circ Res* 1979; 44: 145-53.
 - 8 Thurston TA, Ghusman S. Halothane myocardial depression: interactions with the adenylyl cyclase system. *Anesth Analg* 1993; 76: 63-8.
 - 9 Weinryb I, Michel IM, Alicino JF, Hess SM. Effects of barbiturate derivatives, sulfhydryl reagents, and oxidizing agents on the activity of adenylyl cyclase from guinea pig heart and lung. *Arch Biochem Biophys* 1971; 146: 591-6.
 - 10 Khatter JC, Hoeschen RJ, Dhalla NS. Effects of sodium pentobarbital on rat heart sarcolemma. *Research Communications in Chemical Pathology and Pharmacology* 1979; 24: 57-66.
 - 11 Dan'ura T, Kurokawa T, Yamashita A, Higashi K, Ishibashi S. Inhibition of brain adenylyl cyclase by barbiturates through the effect on the interaction between guanine nucleotide-binding stimulatory regulatory protein and catalytic unit. *Journal of Pharmacobio Dynamics* 1987; 10: 98-103.
 - 12 Dan'ura T, Kurokawa T, Yamashita A, Ishibashi S. Effective inhibition by pentobarbital of forskolin-stimulated adenylyl cyclase activity in rat brain. *Chem Pharm Bull (Tokyo)* 1989; 37: 3142-4.
 - 13 Masssart C, Le Tellier C, Mallédant Y, Leclech G, Nicol M. Modulation of the functional properties of human thyrocytes in monolayer of follicle culture: effects of some anaesthetic drugs. *J Mol Endocrinol* 1991; 7: 57-62.
 - 14 Levine SD, Weber H, Schlondorff D. Inhibition of adenylyl cyclase by general anesthetics in toad urinary bladder. *Am J Physiol* 1979; 237: F372-F378.
 - 15 Blinks JF. Field stimulation as a means of effecting the graded release of autonomic transmitters in isolated heart muscle. *J Pharmacol Exp Ther* 1966; 151: 221-35.
 - 16 Lindmar R, Löffelholz K, Weide W. Inhibition by pentobarbital of the acetylcholine release from the postganglionic parasympathetic neuron of the heart. *J Pharmacol Exp Ther* 1979; 210: 166-73.