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# Thiopentone-induced changes in the contraction pattern of vascular smooth muscle: the influence of albumin

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**1** The influence of thiopentone on (a) resting tension, (b) contractions evoked by exogenous noradrenaline (NA) and (c) contractions elicited by electrical field stimulation of rings of rabbit pulmonary artery was studied over a concentration range limited by the solubility of the drug.

**2** At thiopentone concentrations from $3 \times 10^{-5}$ to $1.4 \times 10^{-3}$ M in a protein-free electrolyte solution (K-H solution) a gradual increase in resting tension to 232% of the control value was observed. The concentration-effect curve was displaced to the left in the presence of albumin (45 g $l^{-1}$) at drug concentrations below $1.6 \times 10^{-4}$ M. Above that concentration the curve was displaced to the right.

**3** The maximal contractile response to exogenous NA in K-H was reduced from 4.1 to 1.8 g by $5.6 \times 10^{-4}$ M thiopentone, but the effects of low concentrations of NA were potentiated by thiopentone. The concentration-effect curve for exogenous NA was displaced to the right by albumin itself.

**4** The contractions evoked by electrical field stimulation in K-H solution were increased by thiopentone up to $2.4 \times 10^{-4}$ M where a maximum of 141% of the control value was reached. Above that a gradual decrease was observed, the height of the contractions being reduced by 75% of the control value at $1.4 \times 10^{-3}$ M thiopentone. Thiopentone failed to potentiate electrically-induced contractions in the presence of albumin K-H, and the concentration-effect curve for the inhibitory effect of thiopentone was displaced to the right.

**5** From relationships between observed responses and free and total drug concentrations, a procedure was suggested to determine a biologically relevant expression for the thiopentone binding to albumin. The biologically determined albumin binding was always less than the binding determined by equilibrium dialysis, indicating that the fraction of thiopentone bound to albumin could not necessarily be considered biologically inactive.

## Introduction

The cardiovascular system is only moderately influenced by anaesthetic doses of thiopentone while higher doses depress the circulation (Conway & Ellis 1969). The total peripheral resistance has been found increased (Flickinger *et al.*, 1961) or unchanged (Dobkin & Wyant, 1957; Dolar & Sun, 1981). In animal experiments a thiopentone-induced fall in sympathetic activity has been demonstrated, and simultaneously a rise in the blood pressure was observed (Millar *et al.*, 1970). Thus a direct effect of thiopentone on the responsiveness of vascular smooth muscle seems possible. Price & Price (1962) studied its effect in the concentration range $9 \times 10^{-5}$ to $3.3 \times 10^{-4}$ M and found an increased response of rabbit isolated aorta to noradrenaline in the presence of thiopentone. We have found no information about the effect of thiopentone over a wider concentration range. In serum, thiopentone is bound to albumin and there is no evidence of binding to other serum proteins (Christensen *et al.*, 1980). However, the effect of thiopentone on isolated vascular smooth muscle has not been studied in the presence of serum proteins.

In the present investigation the effect of thiopentone on rings from rabbit pulmonary arteries was investigated over a wide concentration range. Its interaction with exogenous noradrenaline (NA) and also with electrical field stimulation was studied.

Furthermore, the thiopentone-induced changes in the resting tension of the rings was measured. These effects of thiopentone were investigated both in the absence and presence of albumin.

## Methods

### *Solutions*

Physiological saline (K-H solution) (Krebs & Henseleit, 1932) of the following composition (mM): $Na^+$ 144.2, $K^+$ 5.9, $Ca^{2+}$ 2.5, $Mg^{2+}$ 1.3, $HCO^-_3$ 25.0, $Cl^-$ 126.3, $SO_4^{2-}$ 1.3, $H_2PO_4^-$ 1.2 and glucose 11.1 was prepared in glass distilled water. All reagents were of analytical grade from Merck, Darmstadt, Germany. Some solutions (Alb K-H) in addition contained 45 g bovine albumin per l (crystallized and lyophilized from Sigma). The (−)-noradrenaline tartrate powder was of Nordic Pharmacopoiea purity. The pH of the solutions was monitored by the use of a PHM64 pH-meter and the oxygen tension by a PHM71 Mk 2 (both Radiometer, Copenhagen).

### *Experimental conditions*

The experiments were carried out in thermostatically-controlled tissue baths at 37°C. In rings of rabbit pulmonary artery, changes in resting tension and contractions elicited by NA or by electrical field stimulation were recorded as previously described (Christensen *et al.*, 1982). The pH was adjusted to 7.4 by bubbling the saline with $CO_2$ and the oxygen tension was kept at 700 mmHg. To avoid foam formation in the albumin solutions the gases were added to the tissue bath through a spiral shaped cellophane tubing (Visking 8/32) under pressure (Christensen *et al.*, 1982). Ascorbic acid, 20 mg $l^{-1}$, was added to prevent oxidation of noradrenaline.

### *Experimental procedure*

In all experiments, rings were used of the pulmonary artery removed from albino rabbits (1.8–2.2 kg), killed by cervical dislocation. Para-arterial tissue was removed and the artery was divided in two segments of equal size (about 4 mm). In the planning of experimental series, care was taken to secure an equal distribution between groups of cardiac and peripheral segments. The rings were mounted in the tissue bath at an initial tension of 3 g, adjusted to 1 g after 10 min. An adjustment to 1 g tension was made on each occasion prior to the addition of increasing NA concentrations (at constant thiopentone concentration) or prior to electrical field stimulation (at increasing thiopentone concentrations). Tension in the arterial rings was measured isometrically with a Statham G10B-0.15.350 15 oz transducer and a W + W1200 recorder.

(1) Concentration-effect curves were produced by cumulative addition of NA to the tissue bath. Each incremental dose of NA was added when the effect of the previous dose had almost reached a plateau with less than a 10% rise in tension occurring during a 10 min period. (2) Electrical field stimulation was achieved by a Grass stimulator S88 in connection with a constant current unit, with pulse duration 0.5 ms, frequency 10 Hz, train duration 10 s, voltage 13 V delivered at 10 min intervals. The response to this stimulation was abolished completely by bretylium ($10^{-4}$ M). The first response showing a deviation of less than 10% of the response caused by the previous stimulation was used as the 'control' value. (3) The resting tension was measured immediately before the electrically induced contractions and initial resting tension was that level measured just before the 'control' induced contraction.

The effect of increasing concentrations of thiopentone on the responses (1), (2) and (3) described above was studied in the absence or the presence of albumin. Thiopentone (Leopental sodium) was added cumulatively to the tissue bath at 10 min intervals until the limit of solubility was reached. The 10 min contact period for each thiopentone concentration was chosen because extending the contact period to 20 min was no more effective. During this procedure the pH was periodically adjusted to 7.4. The concentrations of free and total thiopentone in the tissue bath were determined by h.p.l.c. (Christensen & Andreasen, 1979) at all thiopentone levels by equilibrium dialysis (Christensen *et al.*, 1980). No binding of thiopentone to cellophane tubing or the glass bath could be demonstrated.

### *Calculations*

The binding of thiopentone to albumin is reversible and we attempted to assess how far this reversibility influences the equilibrium between unbound thiopentone molecules and thiopentone molecules bound to the vessel.

From our experimental data we constructed curves relating the biological response to: (a) the total thiopentone concentration in a protein solution ($Dt_{(p)}$); (b) the unbound concentration ($Df_{(p)}$ determined by equilibrium dialysis); and (c) the thiopentone concentration in a protein-free K-H solution ($D_{K\text{-}H}$).

The following calculations are based on drug concentrations interpolated from these curves at identical response levels. At a given response level, the equilibrium between thiopentone and albumin can be expressed by an average percentage binding:

$$\frac{Dt_{(p)} - Df_{(p)}}{Dt_{(p)}} \times 100 \qquad (1)$$

Binding becomes almost complete when $Df_{(p)}$ approaches zero while no appreciable binding takes place when $Df_{(p)}$ approaches $Dt_{(p)}$.

A similar equation results if the thiopentone binding is calculated from the size of the biological response. Two assumptions are made: firstly that a fixed relationship occurs between the concentration of free thiopentone and its binding to the vessel and subsequent response; secondly that equal responses in the presence and absence of albumin are caused by equal concentrations of unbound thiopentone. Then,

$$\frac{Dt_{(p)} - Df_{(biol)}}{Dt_{(p)}} \times 100 \qquad (2)$$

where $Df_{(biol)}$ is the biologically-determined free drug concentration. According to the second assumption, $Df_{(biol)}$ is the concentration existing in protein-free K-H at the chosen response level.

Thus equations (1) and (2) when applied to the same response level may be compared, so as to express the biological relevance of the protein binding measured by, for example, equilibrium dialysis.

*Statistical analysis*

The significance of observed differences was tested by the sign test or the Wilcoxon test for paired differences.

## Results

*Stability of the experimental conditions*

Contractions to field stimulation were measured before the addition of thiopentone and again after removal of the drug from the tissue bath (by 10–12 wash-outs) checked by h.p.l.c. analysis of the bath fluid (sensitivity $1.9 \times 10^{-7}$ M). The time periods between the measured pre-drug contractions and post-drug contractions were all close to 130 min. The results in Table 1 indicate that the thiopentone-induced changes in responses to electrical field stimulation are completely reversible in solutions with or without albumin and that no significant difference occurred in the amplitude of contractions in the two solutions. Neither did the presence of albumin affect the resting tension in the tissue.

*Effects of thiopentone*

The influence of thiopentone on resting tension in the absence or presence of albumin is illustrated in Figure 1 which includes for comparison responses of tissues kept equally long under similar circumstances but in the absence of thiopentone. Thiopentone increased the tissue resting tension until a maximum effect of 232% control value was obtained at $1.14 \times 10^{-3}$ M in K-H solution. The increase began at $3 \times 10^{-5}$ M but did not reach a significant value until $1.2 \times 10^{-4}$ M was added. In the presence of albumin the curve was displaced to the right at concentrations of thiopentone above $1.6 \times 10^{-4}$ M and a maximum response of 215% of the control value was reached at $2.4 \times 10^{-3}$ M. At concentrations below $1.6 \times 10^{-4}$ M the curve was displaced slightly to the left; and the increase in resting tension became statistically significant at concentrations from $1.5 \times 10^{-5}$ M in Alb K-H.

As illustrated in Figure 2, thiopentone had a concentration-dependent depressant effect on the maximum response to exogenous NA. The curves indicate that the addition of albumin was without significant effect on the maximum NA-response. A comparison with control tissues not exposed to thiopentone showed that at lower NA concentrations the contractions were potentiated by thiopentone.

**Table 1** Tension changes elicited by electrical field stimulation in rings of rabbit pulmonary artery in K-H solution and in Alb. K-H.

| | *Contractions (g) in K-H solution* ($n = 8$) | *Contractions (g) in albumin solution* (45 g $l^{-1}$) ($n = 8$) | |
|---|---|---|---|
| Before thiopentone | 1.6 (0.7–3.0) | 1.4 (0.8–2.0) | NS |
| After thiopentone | 1.6 (0.8–2.4) | 1.3 (0.8–2.5) | NS |
| | NS | NS | |

The contractions were measured before a cumulative addition of thiopentone and again after a complete wash-out; NS = not significant. Results show mean values and range.

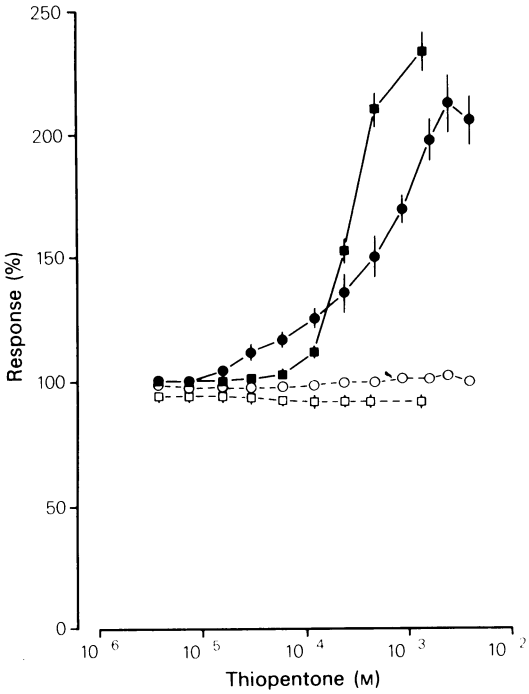


**Figure 1** Changes in the resting tension of rings from the rabbit pulmonary artery as a function of the cumulative thiopentone concentration in K-H solution (■) and Alb.-K-H solution (●). The results obtained under similar circumstances without thiopentone are also shown (K-H solution: □; Alb.-K-H ○). The time interval tween successive additions of thiopentone was always 10 min ($n = 8$). Vertical lines show s.e.mean.

For instance, the presence of $5.6 \times 10^{-4}$ M thiopentone in K-H solution increased the effect of $10^{-8}$ M NA ($P < 0.01$), whilst in albumin K-H solution $4 \times 10^{-5}$ M as well as $3 \times 10^{-4}$ M thiopentone caused potentiation of $10^{-7}$ M NA ($P < 0.05$).

The effect of thiopentone on contractions elicited by electrical field stimulation is illustrated in Figure 3. In K-H solution the electrically-induced contractions were significantly enhanced by low thiopentone concentrations (from $1.5 \times 10^{-5}$ M), a maximum increase to 141% being achieved in the presence of $2.4 \times 10^{-4}$ M thiopentone. At higher concentrations the amplitude of contractions was reduced, by 75% of control values at the maximal obtainable concentration of $1.4 \times 10^{-3}$ M thiopentone. In the K-H albumin solution no significant increase occurred with low concentrations of thiopentone but at concentrations above $8.5 \times 10^{-4}$ M an inhibition of the contractions was seen, a reduction by 30% of control values occurring at $3.8 \times 10^{-3}$ M.

*Biologically determined albumin binding of thiopentone*

The relationship between the measured unbound thiopentone concentration in the presence of albumin and the resting tension is illustrated in Figure 4. For comparison are shown tension changes in relation to the total drug concentration in either albumin K-H solution or in protein-free K-H solution. The curves for unbound drug concentration in the albumin solution are seen to be displaced to the left of the values obtained in the protein-free solution. The

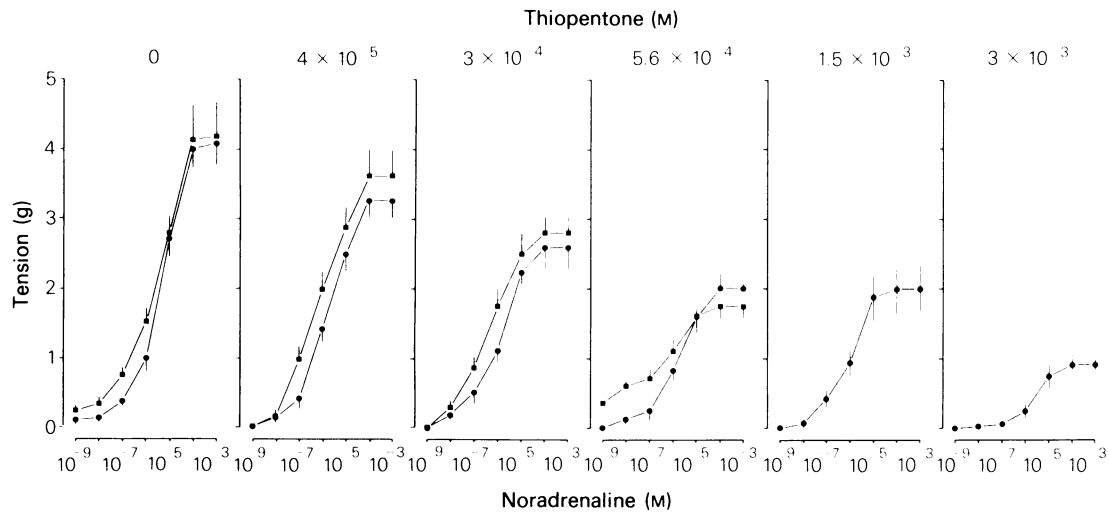


**Figure 2** The increase in tension of rings from the rabbit pulmonary artery as a function of the noradrenaline concentration in the tissue bath. The experiments were carried out in solutions with increasing concentrations of thiopentone and at each level in the presence (●) and absence (■) of albumin. The limited solubility of thiopentone excluded experiments without albumin at the higher thiopentone levels ($n = 8$). Vertical lines show s.e.mean.

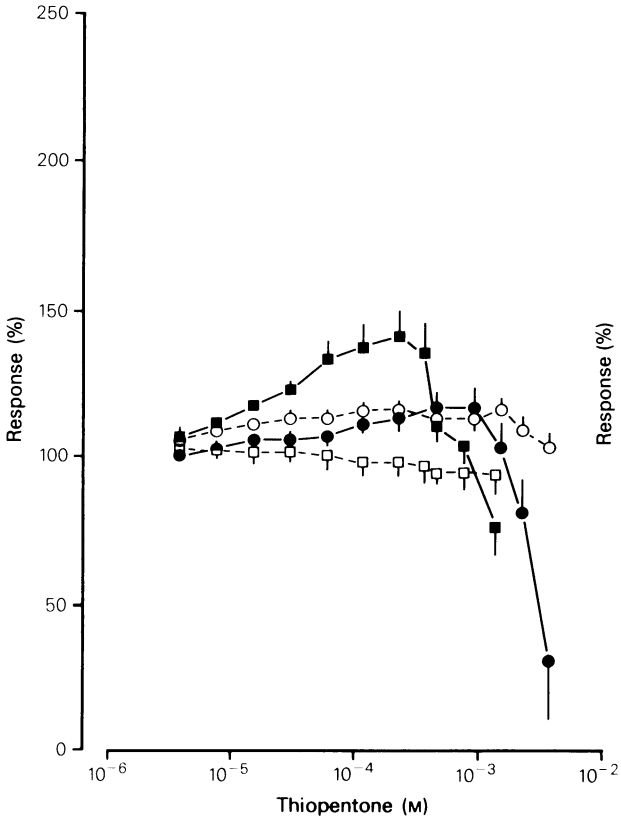


**Figure 3** Changes caused by increasing concentration of thiopentone in contractions elicited by electrical field stimulation in K-H solution (■) and in Alb. K-H (●). The contractions were evoked at 10 min intervals and the time course under similar conditions without thiopentone is also shown (K-H □, Alb. K-H ○). Vertical lines show s.e.mean.

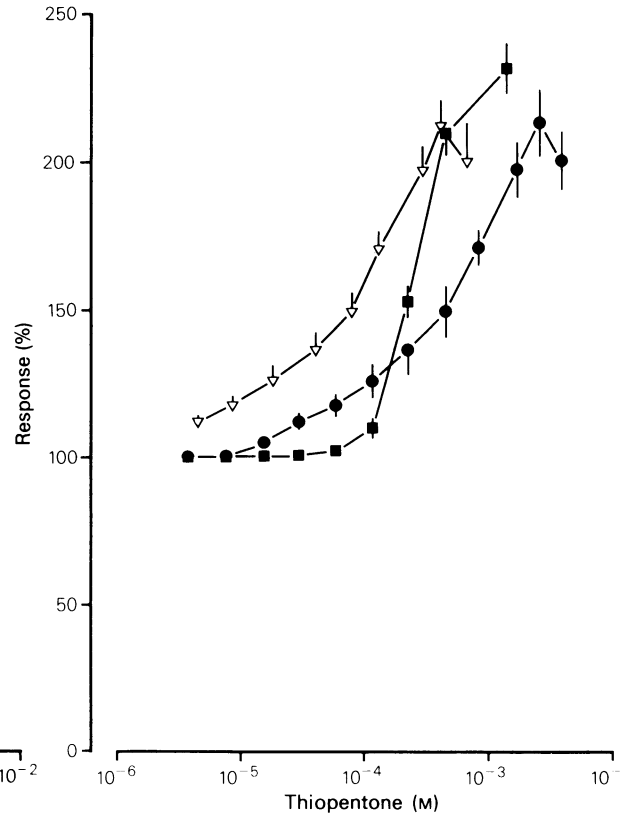


**Figure 4** Changes in the resting tension of rings from the rabbit pulmonary artery as a function of the measured concentration (by equilibrium dialysis) of unbound thiopentone (▽) in Alb. K-H. The relationship between total drug concentration and changes in resting tension is shown (●), and so is the same relationship in a protein-free K-H solution (■) ($n = 8$). Vertical lines show s.e.mean.

**Table 2** The protein binding of thiopentone determined (a) by traditional equilibrium dialysis and (b) biologically, according to equation (2)

| *Response level* (% of maximal increase in tension) | *Total thiopentone concentration in albumin solution* (M) | *% thiopentone bound to albumin* | |
|---|---|---|---|
| | | *Equilibrium dialysis* | *Biologically estimated* |
| 100 | $2.1 \times 10^{-3}$ | 82.8 | 72 |
| | $8.6 \times 10^{-4}$–$2.4 \times 10^{-3}$ | (82.4–83.2) | (30–78) |
| 50 | $4.4 \times 10^{-4}$ | 83.0 | 46 |
| | $1.2 \times 10^{-4}$–$8.6 \times 10^{-4}$ | (82.7–83.5) | (−100–93) |
| 25 | $2.6 \times 10^{-4}$ | 83.3 | 29 |
| | $6.0 \times 10^{-5}$–$8.6 \times 10^{-4}$ | (82.8–83.5) | (−200–79) |
| 10 | $6.8 \times 10^{-5}$ | 83.3 | −78 |
| | $1.5 \times 10^{-5}$–$1.2 \times 10^{-4}$ | (82.8–83.5) | (−700–0) |

Changes (increases) in resting tension were used as the response and the determinations were carried out at the same response level. For further explanation see text (Means and ranges are shown, $n = 8$).

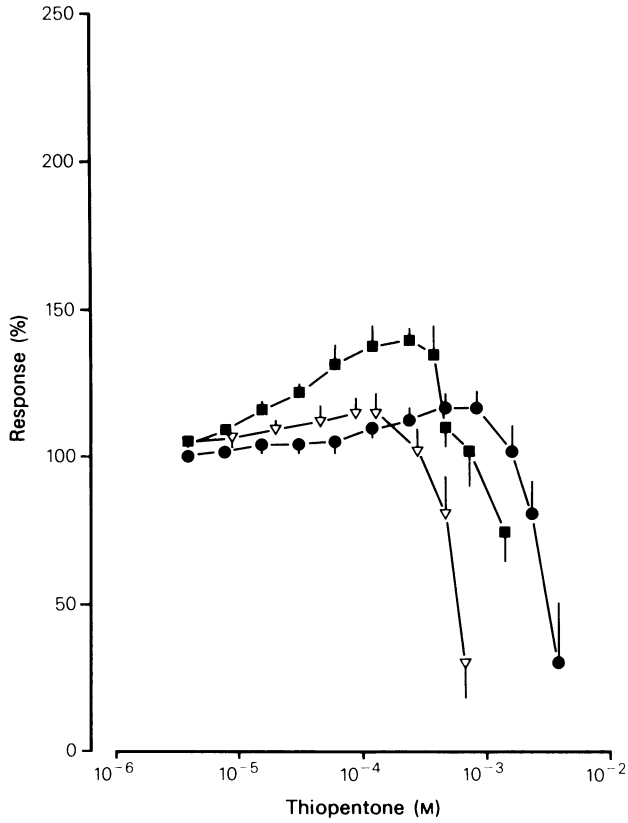


**Figure 5** Changes in contractions elicited by electrical field stimulation of rings of rabbit pulmonary arteries as a function of the measured (by equilibrium dialysis) free concentration of thiopentone in Alb. K-H (▽). The relationship between total drug concentration and changes in contractions is shown (●) and so is the same relationship in a protein-free K-H solution (■). ($n = 8$). Vertical lines show s.e.mean.

approximately parallel curves for unbound and total thiopentone in the albumin solution show that the percentage protein binding is almost constant over the whole concentration range (82.5–83.5%).

Table 2 shows that the biologically estimated degree of binding to albumin (Eq. 2) was lower than the binding measured by equilibrium dialysis. The values were estimated at identical increases in resting tension. The albumin binding determined by equilibrium dialysis was almost constant over the whole concentration range whereas the biologically estimated degree of binding fell progressively with decreasing drug concentrations to reach a negative value below $1.6 \times 10^{-4}$ M.

The relationship between the measured unbound drug concentration in a tissue bath with albumin and the contractions elicited by electrical field stimulation is illustrated in Figure 5. In comparison with the total drug concentration in both albumin K-H solution and protein-free K-H solution In the presence of albumin, unbound thiopentone in concentrations greater than $1.3 \times 10^{-4}$ M caused a significant reduction in the contractions, their being reduced to 30% of the control value at the maximal free drug concentration of $6.8 \times 10^{-4}$ M. This concentration-effect curve for the free drug was displaced to the left of the curve obtained in protein-free K-H solution.

In Table 3 the biologically estimated expressions for the binding of thiopentone to albumin are shown. Again the values are lower than the counterparts obtained by equilibrium dialysis in these experiments using electrical field stimulation.

## Discussion

We examined the effect of thiopentone on rabbit pulmonary artery in three different ways. Price & Price (1962) found an increased sensitivity to ex-

**Table 3** The protein binding of thiopentone determined (a) by traditional equilibrium dialysis and (b) biologically, according to equation (2)

| *Response level* (% of maximal decrease in tension) | *Total thiopentone concentration in the albumin solution* (M) | *% thiopentone bound to albumin* | |
|---|---|---|---|
| | | *Equilibrium dialysis* | *Biologically determined* |
| 25 | $2 \times 10^{-3}$ | 82.5 | 49 |
| | $1.6 \times 10^{-3}$–$2.3 \times 10^{-3}$ | (82.0–83.0) | (36–57) |
| 10 | $1.2 \times 10^{-3}$ | 82.5 | 48 |
| | $8.4 \times 10^{-4}$–$2.3 \times 10^{-3}$ | (82.0–83.0) | (24–73) |

Changes (decreases) in contractions elicited by electrical field stimulation were used as the response and the determinations were carried out at the same response level. Comparable higher response levels than 25% could not be achieved because the limit for thiopentone solubility was reached. For further explanation see text. (Means and ranges are shown, $n = 8$).

ogenous NA ($0.9 \times 10^{-9}$ to $2.4 \times 10^{-8}$ M) of rabbit aorta when thiopentone was present in the solution in concentrations between $9 \times 10^{-5}$ and $3.3 \times 10^{-4}$ M. We also found that the pulmonary artery was more sensitive to low concentrations of NA when exposed to thiopentone but at higher concentrations of thiopentone there was a marked depression of the maximal responses to exogenous NA. The enhancing effect of thiopentone was noted only at low NA concentrations and it is interesting that thiopentone caused an even greater enhancement of electrically-induced contractions and also elevated the resting tension. No definite conclusions about the mechanism by which thiopentone elicits its effects can be drawn from the present results but the thiopentone concentrations necessary for these effects were different. For instance, in order to potentiate the effects of exogenous NA, a concentration of $5.6 \times 10^{-4}$ M thiopentone was needed, whilst resting tension was raised by $1.2 \times 10^{-4}$ M and electrically-induced contractions were enhanced by $1.5 \times 10^{-5}$ M. It is of interest to note that Sevcik (1980), in experiments with giant squid axons, demonstrated that thiopentone ($5 \times 10^{-4}$ M) depolarized the nerve fibres and produced spontaneous repetitive discharges; it is conceivable that thiopentone had both pre- and post-synaptic actions on the pulmonary artery preparation.

In animals of different species Millar *et al.*, (1970) found an increase in blood pressure following the administration of thiopentone 3 mg kg$^{-1}$ body weight. Simultaneously they observed a fall in sympathetic activity. Clinical studies on the cardiovascular effects of thiopentone have resulted in increased (Flickinger *et al.*, 1961) as well as unchanged peripheral resistance (Dobkin & Wyant, 1957; Dolar & Sun, 1981). These *in vivo* studies may reflect the dualistic response to thiopentone which we observed on the pulmonary artery.

*The significance of the presence of albumin*

It was the object of the present study to determine the effect of thiopentone on smooth muscle in the absence and presence of albumin. Three actions of thiopentone were assessed: (1) effects on resting tension; (2) effects on contractions evoked by exogenous NA; and (3) effects on contractions evoked by electrical field stimulation.

It is obvious from Figure 2 that the presence of albumin itself influenced the responsiveness to exogenous NA. Calculations showed a variable binding of NA to albumin; determined biologically from equation 2 the binding of NA was 85% at $10^{-7}$ M, 68% at $10^{-6}$ M and 33% at $10^{-5}$ M. A correction for this variable albumin binding was not attempted, so these experiments were not used to determine the importance of albumin for the thiopentone response.

Table 1 shows that albumin was without significant influence on the electrically-induced contractions when resting tension was adjusted to a baseline of 1 g before each stimulation series. Therefore actions (1) and (3) above were used in attempts to determine the influence of albumin on the effect of thiopentone on smooth muscle. The biologically determined albumin binding (Equation 2) was compared with the binding determined by equilibrium dialysis (Eq. 1). Whether the free fraction of thiopentone is determined by equilibrium dialysis (Dayton *et al.*, 1973; Christensen *et al.*, 1980, 1983) or by ultrafiltration (Andreasen, 1973; Christensen *et al.*, 1980) the fraction is remarkably constant over a wide concentration range. The values of thiopentone binding obtained here are in good agreement with the values which could be anticipated from these previous studies.

The presence of albumin did influence the thiopentone-induced changes in the contraction pattern. At low concentrations, thiopentone had a greater effect in the presence of albumin than in its absence. It is unlikely that the albumin facilitated the transport of thiopentone into smooth muscle cells, because the drug is highly lipophilic, but we have no other explanation for this phenomenon. At thiopentone concentrations above $1.6 \times 10^{-4}$ M, binding in an albumin depot could explain the displacement of the concentration-effect curve for the resting tension to the right. A depot effect could also explain the displacement to the right of the thiopentone-induced decrease in the response to electrical field stimulation. Albumin also reduced the maximum increase in tension caused by thiopentone.

It is frequently stated that only the unbound fraction of a drug is biologically active (e.g. Hug, 1978). In the presence of a binding protein, effective drug concentration at its receptors must depend on two reversible equilibria: one between drug and albumin and one between drug and receptor. It is not possible to characterize the two equilibria by association constants and number of binding sites. An indirect comparison between the two equilibria at a given response level may be achieved, however, by dividing the value from Eq. (2) with the value obtained from Eq. (1). The resulting fraction may be expressed as a percentage indicating the biological relevance of the traditionally determined protein binding. For the thiopentone experiments described in the present study this biological relevance was always less than 100%. Our results indicated that the function of albumin as an inactivating depot may be of some importance at high thiopentone concentrations. For all measured free thiopentone concentrations in albumin solutions the corresponding effect was stronger than would be expected from the corresponding concentration-effect curve in protein-free

K-H solution. So, at least for thiopentone, there seems to be no simple relationship between the size of the biological response and the apparent concentration of unbound drug in solutions containing albumin.

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