ORIGINAL ARTICLE

INTERACTION OF PENTOBARBITAL AND NEUROMUSCULAR BLOCKING DRUGS

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SUMMARY

The effect of pentobarbital sodium was studied in both rat phrenic nerve-hemidiaphragm and rat sciatic nerve-gastrocnemius preparations. It was found that pentobarbital produced twitch depression leading to a complete neuromuscular blockade. The pentoharbital-induced neuromuscular depression was significantly enhanced by d-tubocurarine, succinylcholine and hemicholinium. In chronically denervated muscle, pentobarbital also suppressed acetylcholine(ACh)-contracture. The mechanism of neuromuscular blockade produced by pentobarbital may be (a) reduction of ACh release from motor nerve ending or (b) decrease sensitivity of motor end-plate to ACh.

Several drugs used clinically may have effect on neuromuscular transmission; pentobarbital is one of the barbiturates with such action. In man, the drug is shown to have a rapid onset of action with 1-4 hours duration of hypnotic effect (1). Acute intoxication of the barbiturate may also affect peripheral nervous system. Gross and Cullen (2) showed that the amplitude of stimulus-evoked and ACh-induced contraction in pentobarbital-anesthetized dogs were reduced. The postsynaptic action of barbiturates at neuromuscular junction was reported by Quilliam (3). The curare-like action of pentobarbital was shown in frog nerve-muscle preparation by Thesleff (4) and Riker et al (5). Seyama and Narahashi (6) proposed the presynaptic depressive action of this drug in frog sciatic nerve-sartorius preparation.

In this study, the effect of pentobarbital was investigated in both in vitro and in vivo nerve-muscle preparations in rats. The interaction of pentobarbital and some neuromuscular blocking agents were observed in order to elucidate possible sites and mechanism of action of the drug at this synapse.

MATERIALS AND METHODS

Albino rats weighing about 180-250 gm of either sex were used. The rat was sacrificed by decapitation, hemidiaphragm segments were obtained with the ribs attached. Rat phrenic nerve-hemidiaphragm preparation and the recording of contractile response to neurally evoked twitch were done according to the method of Bübring (7). The directly evoked twitch was recorded in the presence of 5 μM d-tubocurarine.

Rat sciatic nerve-gastrocnemius muscle for recording the neurally evoked contractile response in situ was prepared as described by Rididitid and Apisariyakul (8). In short, the rat was anesthetized with chloralose (100-120 mg/kg, i.p.). A polyethylene tube was inserted into the trachea. The right femoral artery was cannulated with a polyethylene tube filled with heparin in isotonic saline solution (60 units/ml). The inserted part of the cannula was introduced through the right common iliac artery to the bifurcation of the abdominal aorta. A skin incision was made at the mid portion of the left thigh to expose the sciatic nerve. A pair of threads were tied tightly to the main sciatic nerve about 2 mm apart. The nerve was cut between the threads in order to avoid any central connection. The sciatic nerve was kept moist with liquid paraffin. The left Achilles tendon was dissected and tied with a thread attached to a force displacement transducer.

The denervated muscle for recording ACh-contracture was prepared according to Eyzaguirre (9) as modified by Apisariyakul(10). ACh 10 μg/kg body weight was injected intra-arterially to produce ACh-contracture.

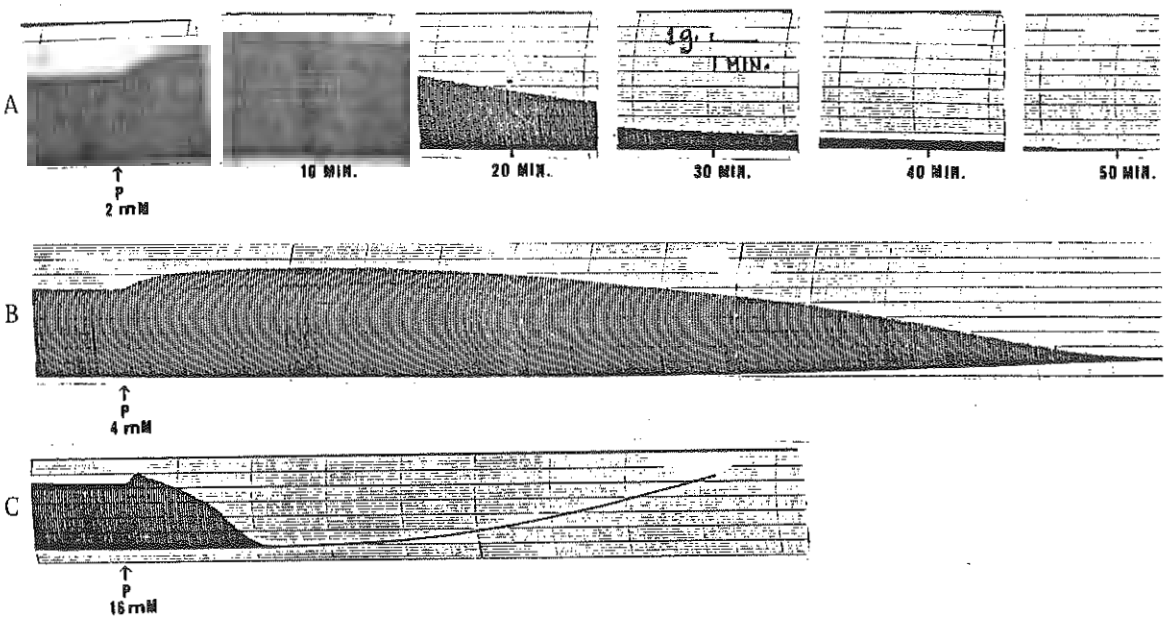

Figure 1. Effect of pentobarbital, 2, 4 and 16 mM on the neurally evoked twitch in the isolated rat phrenic nerve-hemidiaphragm preparation. A, B represent a slight twitch potentiation and gradual decrease in twitch amplitude. C represents a complete neuromuscular blockade.

RESULTS

Neuromuscular effect of pentobarbital

The effect of pentobarbital in the dose of 2,4,16 mM was studied in the isolated rat phrenic nerve-hemidiaphragm preparation. It was found that pentobarbital 2 mM caused a slight increase in twitch amplitude, followed by a gradual decrease in contractile response and finally a complete neuromuscular depression (Figure 1). High concentrations (4 mM and 16 mM) of pentobarbital also produced a complete neuromuscular blockade.

Similar effect of pentobarbital was observed in the sciatic nerve-gastrocnemius preparation in situ.

Interaction of pentobarbital and neuromuscular blocking agents.

When pentobarbital 1 and 2 mM were added to the glass tissue bath containing d-tubocurarine, the percent twitch depression was significantly greater than control ($p < 0.02$, and $p < 0.01$) as indicated in Table 1. In the presence of succinylcholine, the depressive effect of pentobarbital 2 mM on the muscle twitch was significantly increased as indicated in Table 2.

Pentobarbital 2 mM in the presence of hemicholinium (HC_3), a drug which inhibits choline uptake and reduces acethylcholine synthesis (11), produce a complete neuromuscular depression whereas each drug alone did not produce this effect (Figure 2).

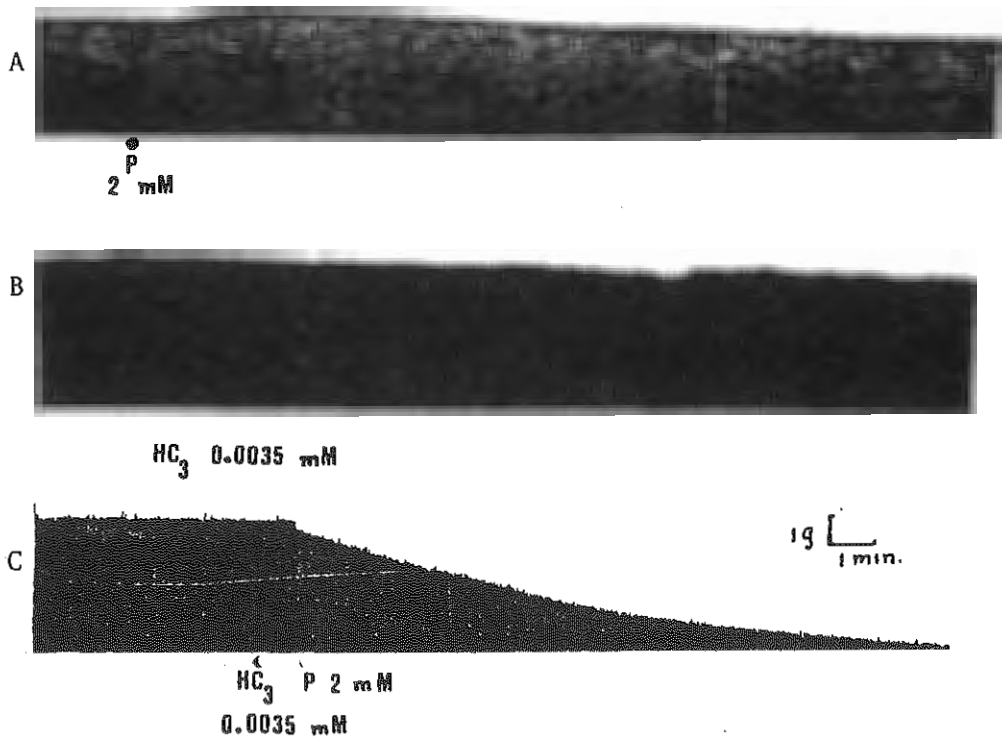

Figure 2. Synergistic effect of 2 mM pentobarbital (P), and 0.0035 mM hemicholinium (HC_3) to produce neuronuscular blockade.
A, B: a slight decrease in twitch amplitude produced by 2 mM pentobarbital, and 0.0035 mM HC_3 ; C: synergistic effect of both drugs.

Table 1 Comparison of the twitch depression produced by d-tubocurarine (d-Tc) and pentobarbital (1 mM. and 2 mM.) in the presence of d-tubocurarine in the isolated rat phrenic nerve-hemidiaphragm preparation.

Dose of d-Tc (mM)	* Percent twitch depression produced by		
	d-Tc (control)	Pentobarbital 1 mM after d-Tc	Pentobarbital 2 mM after d-Tc
0.0008	12.6 ± 1.4	**63.8 ± 9.3	**82.0 ± 10.6
0.0016	70.1 ± 5.8	**90.4 ± 4.3	**97.6 ± 2.4

* Mean of 6 observations at each dose. The twitch depression was measured at 10 minutes after adding d-Tc into the organ bath.

** Significant difference from control ($p < 0.02$).

Table 2 Interaction of succinylcholine (SCh) and pentobarbital, 2 mM in producing twitch depression in the isolated rat phrenic nerve-hemidiaphragm preparation.

Experiments	* Percent twitch depression produced by		
	SCh (0.005 mM) (control)	Pentobarbital (2 mM)	Pentobarbital (2 mM) after SCh
1	16.7	24.5	85.7
2	14.6	10.0	21.0
3	24.1	22.2	62.9
4	8.3	21.8	56.2
5	21.4	19.2	28.4
mean ± S.E.	17.0± 2.8	19.5± 5.6	**50.8± 13.0

* Twitch depression measured at 10 minutes after adding SCh into the organ bath.

** Significant difference from control ($p < 0.05$)

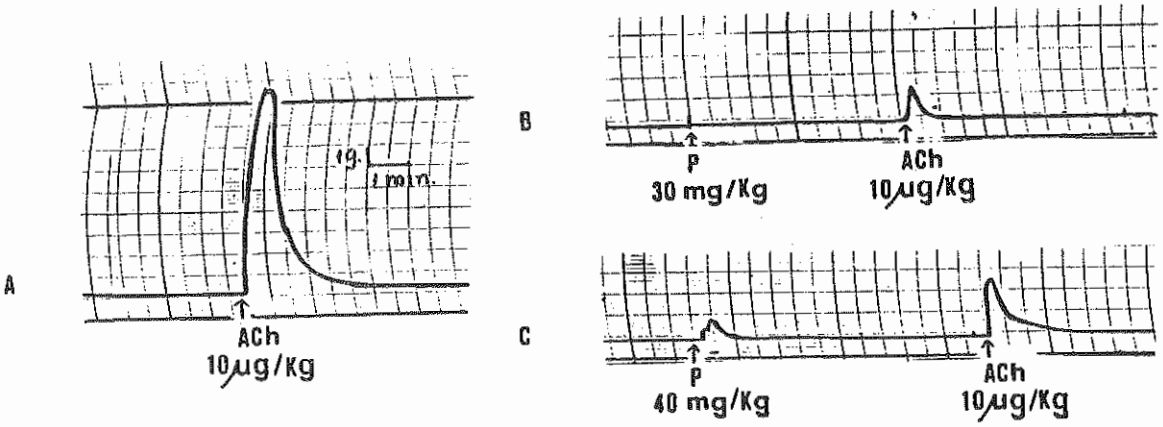

Figure 3. The effect of pentobarbital on ACh-contracture in the denervated rat gastrocnemius muscle preparation. A: control ACh-contracture, B and C: pentobarbital 30 and 40 mg/kg body weight suppressed the amplitude of ACh-contracture.

Effect of pentobarbital on acetylcholine contrature.

In the chronically denervated muscle, the muscle developed sensitivity to ACh (12,13). In this study, intra-arterial injection of ACh 10 μg/kg body weight into chronically denervated rat produced amplitude of muscle contracture about 10 gm as indicated in Figure 3. Pentobarbital in the dose of 30 and 40mg/kg body weight could abolish ACh-contracture.

Comparison of neurally evoked and directly evoked twitch produced by pentobarbital

After adding pentobarbital 2 mM and 4 mM into the tissue bath, the twitch depression of neurally evoked twitch was significantly greater than that of the directly evoked twitch ($p < 0.05$) as shown in Figure 4. It was shown that the twitch depressive effect was primarily due to neuromuscular depression.

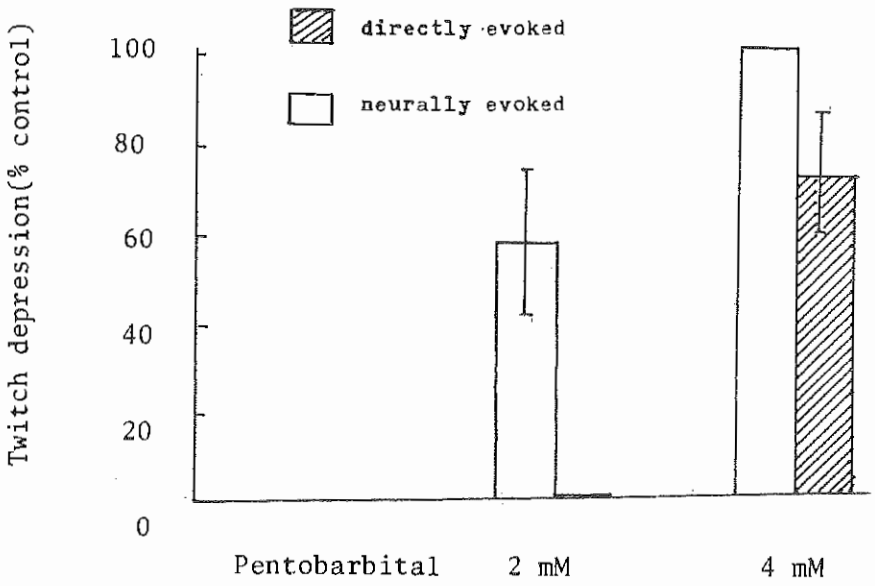

Figure 4. Comparison of neurally evoked (N) and directly evoked (D) twitch depression produced by various doses of pentobarbital at 20 minutes after adding the drug. Asterisk (*) indicates significant difference between neurally evoked and directly-evoked twitch ($p < 0.05$). Peak of each bar is mean of 6 observations ± S.E.

DISCUSSION

Several doses of pentobarbital sodium were studied in both isolated rat phrenic nerve-hemidiaphragm and rat sciatic nerve-gastrocnemius preparation in situ. It was found that pentobarbital in low dose initially produced a slight potentiation and followed by twitch depression, whereas in the high dose only twitch depression was observed. This twitch depression was enhanced by d-tubocurarine, succinylcholine and hemicholinium. Thus, the site of action of this effect may be at the neuromuscular synapse.

It is known that ACh or succinylcholine in low concentrations produce a slight twitch potentiation, and relatively high doses cause neuromuscular depression (14). In this study, it was shown that the

twitch depression produced by pentobarbital was preceded by a slight increase in twitch amplitude which may be considered to be a depolarizing blockade of succinylcholine type; the blockade was potentiated by succinylcholine but not neostigmine (see also 15).

Since the N-trimethylammonium center of the quarternary ammonium compounds seems to be responsible mainly for the interaction of a drug on nicotinic cholinergic recentor at motor endplate to produce direct depolarization like ACh (16, 17). Thus, direct depolarizing action of pentobarbital, which lacks such N-trimethylammonium center on its molecule, on cholinergic receptor is not likely.

ACh-contracture in chronically denervated muscle is a phenomenon indicating a postsynaptic action at neuromuscular synapse (18). The effect of a drug that altered contracture would be an evidence to elucidate possible site and mechanism of action at this synapse. In this study, pentobarbital suppressed ACh-contracture in chronically denervated rat-gastrocnemius muscle. The suppression of pentobarbital on this ACh action implicates the postsynaptic action of the drug. This postulation was in accordance with the work of Quilliam (3) that barbiturate could inhibit ACh-contraction in frog nerve-muscle preparation. In 1970, Adam et al (19) and Thomson and Turkanis (20) also reported that ACh contracture was decreased by barbiturates.

Some experimental results are not in agreement with the view of postsynaptic action of pentobarbital. Hubbard et al(21) and Ricker and Okamoto (22) reported that curare reduced the ACh release from motor nerve ending. In this study, neuromuscular depression by pentobarbital was markedly potentiated by hemicholinium, a drug that inhibits choline uptake to motor nerve terminal and finally leading to the reduction of ACh release (23). The finding that depressive effect of pentobarbital on neurally evoked twitch was significantly greater than that on directly evoked twitch also implicates presynaptic involvement in its action.

In conclusion, the evidence presented in this study supports the postsynaptic as the primary site of action of pentobarbital in producing twitch depression; this may be the result of membrane alterations and a decrease in sensitivity of the motor endplate to ACh. However, the presynaptic action, i.e. the reduction of ACh release from the motor nerve ending, can not be excluded.

ACKNOWLEDGEMENT

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