Age (months)	Body weight (g) Control	Lead-treated	Spleen weight (mg) Control	Lead-treated	Kidney weight Control	Lead-treated	ALAD units/ml erythrocytes Control	Lead-treated	Hematocrit Control	Lead-treated
0.5	36.9±1.0	23.2±3.7	102±13	78± 8	255±77	176±17	17.3 ±0.8	12.5±3.2	39.2±2.1	37.5±1.2
1	60.6±2.2	40.6±1.6	182±6	318±40	344±14	325±13	7.66±0.44	13.7±1.20	40.2±1.0	26.5±2.5
2	153±9	45.5±5.3	337±33	706±126	746±25	608±29	3.75±0.31	11.9±2.30	44.8±1.3	27.5±2.2
3	224±14	125±7	345±25	1190±203	894±52	727±35	3.40±0.46	16.4±7.31	45.2±1.8	26.4±2.6
4	210±12	120±11	340±16	806±135	946±21	802±25	2.98±0.18	19.3±5.4	45.9±2.3	34.7±2.4
7	284±13	125±1.5	354±23	918±60			2.96±0.15	23.3±2.5	46.2±1.9	28.9±2.9
12	296±39	169±12	311±46	1112±155	1126±87	1457±170	3.43±0.67	30.2±0.4	45.3±2.1	22.4±2.7
14 (0.5% Pb)			295±25	952±60	1145±68	2194±280			44.9±1.8	33.2±3.5

Values are means ± SE. All data are from animals given 1% of lead except the incomplete data after 14 months (0.5% lead).

spleen leading to an increase in organ size. More young erythrocytes are therefore present in the blood circulation of lead-treated than in control rats. It may be postulated that ALAD activity, as that of many other erythrocyte enzymes, is only a remnant from the synthesizing stage and diminishes as the erythrocytes age. The greater number of young erythrocytes would thus imply an increased ALAD activity as has indeed been found.

It has long been known that lead shortens erythrocyte survival possibly by damaging their membrane[5,6]. In recent years this aspect has somewhat receded in the general interest, as the depression of blood ALAD and the excess excretion of delta amino levulinic acid focused attention on hem synthesis. Clearly. ALAD in blood cannot be a limiting factor, otherwise anemia would follow the marked depression observed in persons only mildly intoxicated with lead. Moreover ALAD inhibition cannot be complete, otherwise excess urinary excretion and accumulation in erythrocytes of porphyrins could not occur. Indeed, ALAD activity in organs appears much less depressed than it is in blood and under certain conditions may increase (Gerber, unpublished results; Lauwerys, personal communication). The present investigation confirms that, when lead intoxication is severe, survival of erythrocytes becomes the critical factor for changes in blood while ALAD may actually increase in circulating erythrocytes.

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Inhibition of noradrenaline release from sympathetic nerves by pentobarbital[1]

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Summary. Pentobarbital concentrations of 10–100 μM selectively inhibited the noradrenaline release evoked by activation of the nicotinic receptors on the terminals sympathetic nerves of the rabbit heart. Higher concentrations also decreased the noradrenaline release induced by KCl or by electrical stimulation of the nerve axons.

The site and mechanism of action of barbiturates on the nerve cell is not yet known. Previously, in-vitro studies provided evidence that barbiturates are capable of inhibiting Na^+ conductance[3,4] and Ca^{2+} permeability[5,6] of the cell membrane; however, the concentrations which are necessary for this inhibition would cause severe poisoning in vivo. In the present study, the terminal sympathetic nerves of the rabbit heart were used as a model for the investigation of the membrane actions of the drugs.

Methods. The experiments were made on isolated hearts of rabbits (either sex) weighing 1.6–2.8 kg. All details of the methods used have been described previously[7]. Briefly, the hearts (some of them with an intact postganglionic sympathetic nerve supply[8]) were perfused with Tyrode solution (33 °C) at a constant flow rate of 25 ml/min. The composition of the solution was as follows (mM): NaCl 137; KCl 2.7; $CaCl_2$ 1.8; $MgCl_2$ 1.1; $NaHCO_3$ 11.9; NaH_2PO_4 0.4; glucose 5.6; ascorbic acid 0.06 (aeration with 95% oxygen and 5% carbon dioxide). The noradrenaline concentration in the perfusate was measured spectrofluorimetrically by a modification of the trihydroxyindole method. In addition, heart rate and tension developed by the hearts were determined.

Results and discussion. Pentobarbital at concentrations up to 1 mM neither significantly altered the spontaneous noradrenaline output, nor the ability of the heart to remove exogenous noradrenaline (table). Since most of the exogenous noradrenaline removed during the passage through the coronary vessels of the rabbit heart is taken up into the noradrenergic neurons[9,10], we conclude that pentobarbital does not inhibit the noradrenaline uptake into the cardiac sympathetic nerves. Hence, changes of noradrenaline output from the hearts caused by this drug are due to alterations of noradrenaline release from the nerves.

Figure 1 shows that pentobarbital at concentrations up to 100 μM selectively inhibited the noradrenaline release evoked by activation of the nicotinic receptors with acetylcholine (muscarinic receptors blocked with atropine); there is evidence that such receptor sites exist in the mem-

brane of the terminal sympathetic nerves[11–13]. The pentobarbital concentration which caused 50% inhibition of the noradrenaline release (IC_{50}) induced by acetylcholine amounted to 34 μM. In order to evaluate the type of inhibition caused by pentobarbital, concentration-response curves of acetylcholine (in the presence of atropine) were determined. As shown in figure 2, pentobarbital decreased the maximum effect of acetylcholine in a concentration-dependent manner, indicating that the barbiturate behaved like a noncompetitive antagonist. Pentobarbital concentrations higher than 100 μM also decreased the noradrenaline release in response to 80 mM KCl or to electrical stimulation of the nerve axons (figure 1; IC_{50}: 190 and 440 μM, respectively). All changes of noradrenaline release shown in figure 1 were reversible within 10 min after withdrawal of the drug. Heart rate and peak tension developed by the hearts were not at all affected by pentobarbital concentrations up to 100 μM; a concentration of 1 mM decreased heart rate and tension by 26 and 85%, respectively.

All methods of stimulation used in the present study induce Ca^{2+} influx into the terminal sympathetic nerves, which is the vital link between stimulation and noradrenaline release[7,8,14,15]. Hence, high concentrations of pentobarbital which inhibit the noradrenaline release in response to all methods of stimulation may cause this effect by decreasing the Ca^{2+} permeability of the membrane. This suggestion is supported by the finding that these barbiturate concentrations are capable of blocking Ca^{2+} uptake by depolarized nerves[5,6].

The chain of events that are initiated by binding of acetylcholine to the nicotinic receptors, or by electrical stimulation of the nerve axons, are well known; both methods of stimulation induce depolarization of the terminal sympathetic nerves and Ca^{2+} influx which in turn cause

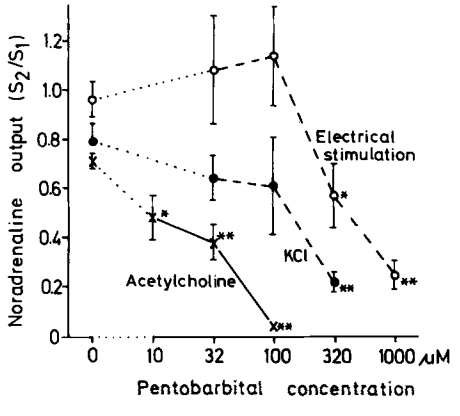

Fig. 1. Inhibition by pentobarbital of the stimulated noradrenaline output from the isolated rabbit heart. Each point represents the mean (± SEM) of 4–9 experiments. Methods of stimulation used: 1. electrical stimulation of the nerve axons (○; square wave pulses of 3 msec duration and supramaximal current strength at a frequency of Hz; each stimulation period lasted for 1 min: the right and left nerves were stimulated alternately, each side twice for 15 sec); 2. stimulation by raising the KCl concentration in the perfusion fluid by 80 mM for 2 min (●); 3. stimulation with acetylcholine, 180 μM, for 30 sec (×; in the presence of atropine, 3.5 μM). Each preparation was stimulated 3 times (S_1–S_3) at intervals of 15 min (1st stimulation period 20 min after the preparation had been set up). All values indicate the output evoked by S_2 (pentobarbital present 10 min before and during S_2). The noradrenaline output was expressed as the fraction of that evoked by S_1. The noradrenaline output evoked by S_1 (all experiments shown in the figure) amounted to: 46 ± 4 ng/2 min (N = 31, electrical stimulation); 153 ± 21 ng/2 min (N = 21, stimulation with KCl); 931 ± 87 ng/2 min (N=22, stimulation with acetylcholine). *$p<0.05$; **$p<0.005$.

1 This paper is dedicated to Professor Dr. G. Malorny on the occasion of his 65th birthday.
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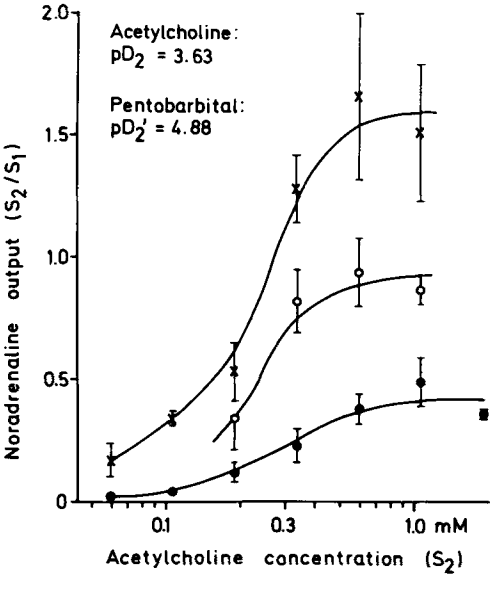

Fig. 2. Effect of pentobarbital on the concentration-response curve of acetylcholine (in the presence of atropine, 3.5 μM) for its stimulating effect on the noradrenaline output from isolated rabbit hearts. Desipramine, 150 nM, was present in the perfusion fluid throughout the experiment in order to inhibit the neuronal reuptake[18]. Each preparation was stimulated with acetylcholine 2 times (S_1 and S_2) for 30 sec (interval between S_1 and S_2 15 min). During S_1 the acetylcholine concentration was 0.18 mM, whereas during S_2 it varied between 0.058–1.84 mM. All values indicate the output evoked by S_2, either in the absence of a barbiturate (controls:x) or in the presence of pentobarbital (10 μM:○; 32 μM:●; 10 min before and during S_2). The noradrenaline output evoked by S_2 was expressed as the fraction of that evoked by S_1. The noradrenaline output induced by S_1 (all experiments shown in the figure) amounted to 465 ± 37 ng/2 min (N = 67). Each point represents the mean (± SEM) of 3–5 experiments. The pD_2 value of acetylcholine and the pD_2 value of pentobarbital against acetylcholine were determined as described by Van Rossum[19].

noradrenaline release by exocytosis[16]. Hence, it may be concluded that the selective inhibition of acetylcholine-induced noradrenaline release caused by low pentobarbital concentrations is due neither to an impairment of the exocytotic release mechanism per se, nor to an inhibition of depolarization, nor to a decrease in Ca^{2+} inward current. The latter conclusion implies that Ca^{2+} influx occurs via unspecific Ca^{2+} channels which can be opened by all methods of stimulation used. However, the possibility must be considered that specific Ca^{2+} channels are opened by activation of the nicotinic receptor. This receptor is a highly hydrophobic protein which traverses the lipid matrix of the membrane[17]; both the binding site for acetylcholine and the ionophore involved in the translocation of ions are localized and coordinated within this macromolecule[17]. Evidence has been presented that barbiturates are able to cause a conformational change of membrane proteins[4]. Taken together, we conclude that these drugs may induce a conformational change of the nicotinic receptor which may either block the gating mechanism for the opening of specific Ca^{2+} channels, or prevent the interaction of acetylcholine with the receptor, thus inhibiting stimulus formation. This conclusion is consistent with our finding that pentobarbital causes a non-competitive inhibition of the effect of nicotinic receptor stimulation.

Influence of pentobarbital on the spontaneous noradrenaline output from isolated rabbit hearts and on the removal of exogenous noradrenaline from the perfusion fluid

Pentobarbital concentration (mM)	Noradrenaline output (ng/2 min)*	Removal of noradrenaline (% of the amount infused)**
0	2.8 ± 1.6	41.0 ± 3.5
0.32	3.3 ± 2.3 (n.s.)	47.0 ± 8.2 (n.s.)
1.0	3.9 ± 1.1 (n.s.)	48.4 ± 4.4 (n.s.)

Means ± SEM (N = 5–10). n. s., not significantly different from controls. *Pentobarbital was present in the perfusion fluid 8 min before and during sampling of the perfusates. **Noradrenaline was infused into the aortic cannula for 10 min to give a final concentration of 59 nM. Pentobarbital was present 10 min before and during noradrenaline infusion.

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Effect of carbenoxolone on phosphodiesterase and prostaglandin synthetase activities[1]

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Summary. Carbenoxolone inhibited in vitro cAMP and cGMP phosphodiesterases in a concentration-dependent and noncompetitive manner. Prostaglandin synthetase activity of rabbit kidney medulla was slightly stimulated by carbenoxolone 0.1–0.5 mM, but inhibited by higher concentrations.

Glycyrrhizic acid is one of the numerous substances which have been extracted from liquorice root. Its aglycone is glycyrrhetinic acid, from which carbenoxolone sodium is synthesized. Carbenoxolone has been used in the treatment of gastric and duodenal ulcers[2,3], and it was the first drug convincingly shown to accelerate the rate of healing of chronic gastric ulcer[4]. The mode of action is uncertain, but it seems likely that the drug increases the defensive reactions of the stomach by stimulating or by altering the physical characteristics of mucous secretion[5,6]. The clinical use of carbenoxolone is limited by side-effects due to salt and water retention and potassium loss.

Cyclic adenosine-3′,5′-monophosphate (cAMP) has been suggested to be an intracellular mediator of histamine-induced acid secretion[7], and cyclic guanosine-3′,5′-monophosphate (cGMP) seems to participate in pentagastrin-stimulated acid formation[8,9] Prostaglandins (PGs), on the other hand, have been suggested to function as a physiological brake in the gastric secretion[10]. On this basis, it seemed important to study the effect of carbenoxolone on these agents.

Materials and methods. Phosphodiesterase activities of the fundus part of rat stomach were measured using ^{3}H-cAMP or ^{3}H-cGMP as substrates according to the method of Thompson and Appleman[11]. The inhibitory effects of drugs were measured in duplicate at 5–6 different substrate concentrations (0.1–2.0 μM). K_m, V_{max} and K_i values were calculated from the double reciprocal plot of the Michaelis-Menten equation.

Rabbit kidney medulla has a high PG synthetase activity and is routinely used for evaluating PG formation. In the present study, microsomal fraction of rabbit kidney medulla was used as an enzyme source[12] for measuring the effect of carbenoxolone on PG synthetase. PGE was determined on superfused hamster stomach strip[13].

Drugs and chemicals. ^{3}H-cAMP (27.5 Ci/mmole) and ^{3}H-cGMP (21 Ci/mmole) were supplied by The Radiochemical Centre, Amersham, England. Arachidonic acid (99%) and bovine serum albumin (Sigma Chemicals Co, St. Louis, Mo., USA), hydroquinone (Fluka AG, Buchs, Switzerland), reduced glutathione (E. Merck, Darmstadt, Federal Republic of Germany), carbenoxolone sodium (MS Chemicals, Milano, Italy) and theophylline (pH. Nord.) were used.

Results. Phosphodiesterase. The K_m-values for rat gastric mucosa phosphodiesterase were 1.3 μM for cAMP and 2.0 μM for cGMP. Carbenoxolone concentration-dependently (50–100 μM) inhibited the phosphodiesterases for cAMP and cGMP. The inhibitor constant (K_i) for cGMP phosphodiesterase was 0.22 mM. The type of inhibition was noncompetitive (figure 1). The noncompetitive type of cAMP phosphodiesterase inhibition described by Amer et al.[14] was only once obtained. The inhibitor constant was then 0.042 mM. Other experiments gave the results plotted in figure 1.

Carbenoxolone seemed to activate cAMP degradation at high substrate concentrations. A strong substrate inhibition of cAMP phosphodiesterase was obtained at