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Some Pharmacologic Properties of Pangamic Acid (Vitamin B-15).* (26644)

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Pangamic acid (dimethyl-amino-acetyl-gluconic acid) also known as Vit. B-15, was discovered by E. T. Krebs in 1938. The presence of this vitamin has been demonstrated in apricot kernels, rice bran, brewer's yeast, ox blood and horse liver. It is thought to accompany the other members of the Vit. B complex(1,2). Although pangamic acid has been reported to be without pharmacologic properties, it has been suggested for use in treatment of cardiovascular and rheumatic diseases, alcoholism and liver cirrhosis(1. 2,3). This investigation deals with some pharmacologic properties of pangamic acid. Although this compound appears to be structurally dissimilar from thiamine, this investigation demonstrates that pangamic acid shares some of the pharmacologic actions of thiamine(4,5,6).

Methods. Neuromuscular blocking activity was evaluated by using the sciatic nerve-gastrocnemius muscle preparation in the rabbit and chicken. Mature, Dutch rabbits were anesthetized with sodium phenobarbital (200 mg/kg) and bipolar, silver electrodes were placed on the peripheral end of the sectioned sciatic nerve. The nerve was stimulated for 0.2 second with monophasic, square wave pulses of 2 milliseconds duration at a frequency of 250 per second and of supramaximal voltage delivered every 5 seconds. A Grass stimulator (Model S4C) with circuit interrupter was used in all experiments. Semi-isometric recordings were made on smoked kymograph paper. Sixteen animals were used to evaluate the activity of pangamic acid. A linear regression and the dose producing 50% neuromuscular blockade (ED-50) and its 95% confidence interval were calculated as outlined by Finney(7). The neuromuscular effects of pangamic acid were also studied in the chicken using the method described by Pelikan *et al.*(8). The parameters described for the rabbit sciatic nerve-gastrocnemius muscle preparation were also used in this preparation.

The effect of pangamic acid on blood pressure was studied in 20 anesthetized dogs (thiopental soduim 15 mg/kg and barbital sodium 250 mg/kg). Carotid or femoral arterial pressure, electroencephalographic (EEG) and electrocardiographic (ECG) recordings were made. In 5 dogs, the left fore limb was perfused with blood from the abdominal aorta using a constant flow Sigmamotor pump. Injections were made into the perfusing blood or given systemically. Systemic arterial blood pressure and fore limb perfusion pressure were recorded simultaneously. Drug action on the isolated rabbit heart was studied by the clas-

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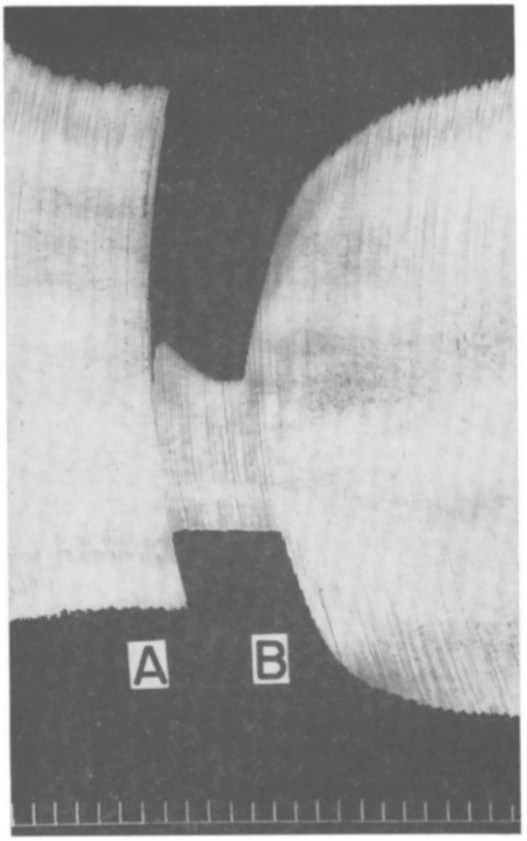

FIG. 1. Effect of neostigmine methylsulfate upon neuromuscular blockade produced by pangamic acid. Time marks equal 1 min. A, pangamic acid, 100 mg/kg. B, neostigmine methylsulfate, 50 μg/kg.

sical method of Langendorf(9). The effect of pangamic acid on spontaneous contractions of isolated rabbit ileum was also studied. Solutions of pangamic acid (Nutritional Biochemicals) were freshly prepared and administered intravenously in the neuromuscular and blood pressure studies.

Intravenous and intraperitoneal LD-50 values were obtained by determining the effect of at least 3 dose levels yielding between 10 and 90% effect. At least 10 mice (Rockland strain, Sutter Farms) were utilized in determining each point. Computations for fitting the probit-log dose regression lines determining the LD-50 and 95% confidence intervals were done according to the method of Finney(7).

Results and discussion. Toxicity studies in mice showed that the LD-50 for pangamic acid following intraperitoneal administration was 1349 mg/kg with 95% confidence limits of 1148 to 1514 mg/kg. This value is considerably lower than that reported by Krebs *et al.*(1) and Millet(2): 14,700 ± 650 mg/kg. The LD-50 value following intravenous administration was found to be 255 mg/kg with 95% confidence limits of 170 to 513 mg/kg. The mice died of respiratory paralysis as evidenced by the fact that upon opening the chest cavity the heart continued to beat.

The dose of pangamic acid causing 50% neuromuscular blockade in rabbits was 129 mg/kg with 95% confidence limits of 126 to 135 mg/kg. The blockade was rapid (1-2 min) in onset and reversible; the duration of blockade being dose related. Neuromuscular blockades of 70-80% returned to control levels within 2 hours. Neostigmine methylsulfate was found to be an effective antagonist of the blockade (Fig. 1). In the chicken, pangamic acid produced a flaccid paralysis which suggests a non-depolarizing type of blockade.

Intravenous administration of pangamic acid in anesthetized dogs resulted in a rapid depressor response of variable duration. A dose of 5 mg/kg (8 dogs) produced a fall in blood pressure of 24 ± 7 mm of mercury. A slight sinus bradycardia was the only observable change found in the ECG. Blood pressure responses of small doses of epinephrine, levarteranol, acetylcholine, histamine and tetramethyl ammonium measured before and after administration of pangamic acid did not differ markedly. The pressor response to bilateral carotid occlusion before and after drug was not altered. Neither vagal section nor administration of atropine sulfate (1 mg/kg), scopolamine hydrobromide (1 mg/kg), chlorprophenpyridamine maleate (25 mg/kg) nor promethazine hydrochloride (25 mg/kg) altered the magnitude of the depressor response produced by pangamic acid.

Fore limb perfusion studies indicated that peripheral vasodilatation does not contribute markedly to the depressor response seen when pangamic acid is administered intravenously.

In the isolated rabbit heart, pangamic acid

in doses up to 20 mg dissolved in Locke-Ringer solution and injected into the coronary inflow cannula produced only slight, transient depression of contractions and some reduction of rate.

Concentrations of pangamic acid up to 6 $\times$ 10^{-4} M did not alter spontaneous contractions of the isolated rabbit ileum, nor did this concentration of drug modify contractions produced by acetylcholine.

Our data demonstrate that pangamic acid is not without pharmacologic effect. We have found the LD-50 in mice after intraperitoneal administration to be much lower than the value previously reported for this compound. It has been reported that thiamine hydrochloride possesses neuromuscular blocking activity and that this effect is antagonized by neostigmine methylsulfate(4,5, 6). It has also been demonstrated that intravenous administration of thiamine hydrochloride produced a transient but marked hypotension with concomitant bradycardia in anesthetized dogs. In these respects, pangamic acid produces pharmacological effects not unlike those of thiamine hydrochloride. Circulatory collapse has been reported clinically from intravenous and intramuscular injections of thiamine hydrochloride(10). We are not aware of any clinical reports of respiratory depression or severe hypotension resulting from administration of pangamic acid. However, it is conceivable that pangamic acid could produce undesirable clinical effects under circumstances of overdosage.

Summary. Pangamic acid (Vit. B-15) possesses definite pharmacologic properties. These include neuromuscular blocking activity in the rabbit and chicken as well as production of hypotension in anesthetized dogs. Neostigmine methylsulfate is an effective antagonist to the neuromuscular blockade produced in the rabbit. These effects appear to be qualitatively similar to those reported for thiamine hydrochloride.

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Leukokinetic Studies V. Uptake of Tritiated Diisopropylfluorophosphate by Leukocytes.* (26645)

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The use of radioactive diisopropylfluorophosphate (DFP^{32}) as a label for leukocytes has been described in previous reports from this laboratory(1-3). Leukocytes have been labeled *in vitro* and returned to the circulation of the donor(2), or they have been labeled *in vivo* by administering the DFP^{32} intravenously(1,3). During these studies it was demonstrated that polymorphonuclear (PMN) neutrophils but not lymphocytes are

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