The Effects Produced by the Interaction between Potassium Ion and Pentobarbital on the Force of Contraction of Isolated Guinea Pig Atria* (33808)

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(Introduced by R. K. Richards)
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Both pentobarbital and increasing potassium ion concentration depress myocardial contractility. The effects produced by the interaction of these agents has not been investigated. The purpose of this study was to investigate the interaction of potassium and pentobarbital on a simple one-variable system, the peak force of contraction of isolated guinea pig atria, and to use the results to draw rudimentary conclusions concerning the mechanism of action of these agents.

Methods. Six guinea pigs, 300–500 g of body weight, were killed by concussion. The heart was quickly removed for further dissection. An isolated atrial preparation was set up as described previously (1, 2). The preparations, contained in a 50-ml bath of Krebs' solution, pH 7.40–7.43 and 28°, were stimulated with punctate platinum electrodes by square wave pulses of 1–5 V, 5 msec duration, at a frequency of 120–180 /min. Isometric contractions were recorded by a Sanborn FTA-3 transducer.

The following separate studies were performed in random sequence on each preparation.

1. Pentobarbital sodium[2] (0.25%) was added to the bath in doses of 0.1 or 0.2 ml, producing increments of 0.5 or 1 mg/100 ml (4×10^{-5} or 8×10^{-5} *M*).

2. Potassium chloride, 0.25 *M* solution, was added in doses of 0.2 ml, producing increments of 0.5 mmoles/liter.

3. Pentobarbital sodium was added in increments of 0.1 ml or 0.2 ml together with potassium chloride in increments of 0.1 or 0.2 ml.

After each dose, 5 min were allowed for the full response to develop. Following each of the above subexperiments, the bath was flushed several times with fresh Krebs' solution and 45–60 min were allowed for the preparation to stabilize before the next run was begun.

In this study, synergism (potentiation) is defined as an effect of a combination of agents which is greater than that which would be predicted from the effect of the sum of the drugs, as defined by the dose-additive interpretation of interaction (3). Antagonism is an effect of a combination of agents which is less than the effect predicted from the sum of the drugs.

To determine the effects of sums of pentobarbital and potassium ion and their relation to the effects of combinations of the two agents, isobolograms were constructed by a method described earlier (3, 4). In brief, concentration–effect curves were constructed in each experiment for each drug alone. Lines parallel to the concentration axis were drawn through the concentration–effect curves. The values of these lines were not selected by the investigators, rather the height of each line was determined by the effect produced by a combination of the two agents administered simultaneously (Fig. 1). The points of intersection of each line with the two concentration–effect curves were transferred to a new graph with linear coordinates (concentration of potassium versus concentration of pentobarbital, Fig. 2). Straight lines drawn between the pairs of points established the additive isobole. Points representing the combined concentrations of the two drugs were then plotted. If a point fell on its

* Supported in part by USPHS Program Project No. GM 12527-04 and Army Grant No. DA-49-193-MD-2135.

[1] Recipient of NIH Career Development Award No. 5-K3-GM-31757-03.

[2] Prepared no earlier than 15 min prior to an experiment by dissolving crystalline material in deionized distilled water.

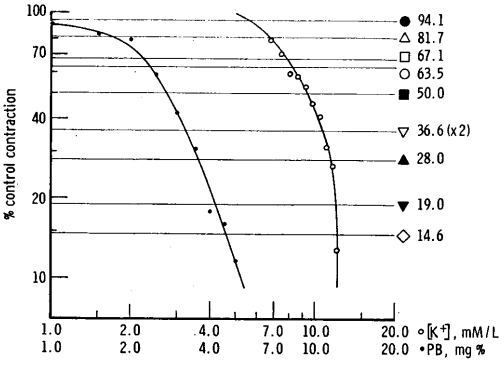

FIG. 1. Percentage of control peak force of contraction of isolated guinea pig atria against concentrations of potassium ion and pentobarbital: equal-effect lines have been drawn through the concentration–effect curves at 94.2, 81.7, 67.1, 63.5, 50.0, 36.6, 28.0, 19.0, and 14.6% of control contraction. Note that two combinations of drugs produced a 36.6% effect. One mg/100 ml of pentobarbital = 8 × $10^{-5}M$ concentration.

corresponding isobole, an additive effect was considered present; points falling toward the origin indicated synergism; points falling away from the origin indicated antagonism.

Results and Discussion. Either adding pentobarbital or increasing the potassium ion concentration decreased the peak developed tension of the atria at all concentration levels. Figure 1 illustrates the concentration–effect curves from one preparation, plotting pentobarbital and total potassium ion concentration against the percentage of control peak developed tension. A number of equal-effect lines have been drawn through the concentration–effect curves. Each line represents the effect of a different combination of concentrations of pentobarbital and potassium.

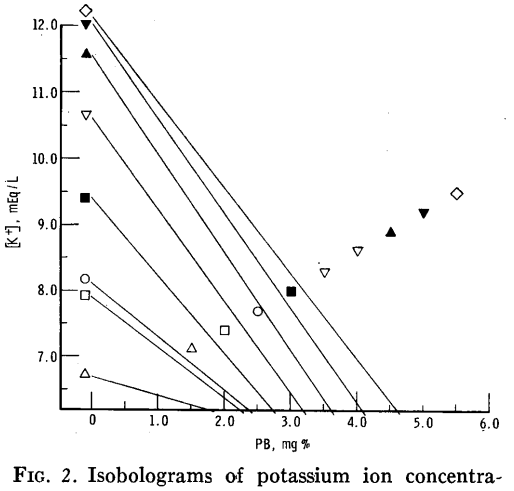

FIG. 2. Isobolograms of potassium ion concentration against pentobarbital concentration: additive isoboles connect each pair of points representing the interaction of the pentobarbital and potassium concentration–effect curves with their predetermined effect planes.

Figure 2 illustrates the isobolograms constructed from the pentobarbital–potassium ion curves in Fig. 1. An additive isobole connects each pair of points representing the intersection of the pentobarbital and potassium concentration–effect curves with a corresponding effect plane. The combined concentration of pentobarbital and potassium which produces a given depression in peak developed tension is plotted with its corresponding symbol. Thus, the closed square corresponds to the 50% effect plane (Fig. 1), additive isobole (Fig. 2), and concentrations of 8.09 mmoles/liter of potassium and 3 mg/100 ml ($2.4 \times 10^{-5}\ M$) of pentobarbital (Figs. 1 and 2). All points fall well above their corresponding additive isoboles, indicating antagonism between the myocardial depressant effects of pentobarbital and potassium. At moderate levels of depression (98.3—36.6% of control peak developed tension) this antagonism is of the type previously termed "iso-antagonism" (3), that is, the effect of a combination of agents in certain fractions of equi-effective doses is equal to the effect of either drug alone. At the higher effect levels (< 36.6% of control peak developed tension) the antagonism is "supra-antagonism", that is, the effect is less than the effect of either agent alone. Antagonism was demonstrated in each of the six atria. The shift from isoantagonism to supra-antagonism was seen at a mean of 40.2% effect level (± 4.1% SD).

The depressant action of pentobarbital on the myocardium has been known for decades. The mechanism of action, however, remains obscure, in spite of numerous suggestions. It has been postulated that pentobarbital chelates calcium or in some manner alters its metabolism, thereby depressing excitation–

contraction coupling (5). Other workers have suggested that removal of calcium from its membrane site provides a site of attachment of sodium to this site, thereby producing a loss of potassium from the cell (6). Experimentally, it has been demonstrated that pentobarbital induces a decrease in potassium conductance; this decrease has been used to explain the elevation of the threshold of the fiber to direct electrical stimulation and the reduction or elimination of the action potential (7). None of these past reports have attempted a satisfactory explanation for their reported data. All imply a vague relationship between pentobarbital, potassium, and calcium. Our results similarly confirm a relationship between pentobarbital and potassium, but they permit few conclusions to be drawn. Isoantagonism implies that one agent uncouples the myocardial depressant action of the other, rendering it inert. Competition for a common receptor site would be an explanation for supra-antagonism.

Summary. The interaction between pentobarbital and increasing potassium ion concentration was investigated in isolated guinea pig atria. Each agent administered separately depressed the peak developed force of contraction. Combinations of the agents, as evaluated by isobolograms, produced isoantagonism at moderate effect levels, supra-antagonism at higher effect levels.

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Received Nov. 6, 1968. P.S.E.B.M., 1969, Vol. 131.

A Comparison of Fetal versus Maternal Plasma Colloidal Osmotic Pressure in Man* (33809)

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The colloidal osmotic pressure (COP) measurements of maternal and fetal plasmas in sheep and goats have shown that the COP of the maternal plasma is higher than that of fetal plasma throughout gestation and that the magnitude of the difference is greater early in gestation (1). In man similar data are not available although McCarthy (2) reported COP measurements on diluted fetal plasma at term. In this study we have attempted to measure the total COP on undiluted plasma of paired human maternal and fetal plasmas collected at different stages of gestation. In addition, the discription of a rigid membrane micro-osmometer is given.

* Supported by USPHS Grants HD 00781 and HD 01632.

Materials and Methods. In 33 uncomplicated full-term pregnancies in man, paired samples of fetal and maternal blood were obtained within 5 min after delivery. Fetal blood was obtained from the umbilical cord and maternal blood was obtained from the antecubital vein. Nine paired samples of maternal and fetal blood were obtained between 26 and 36 weeks of gestation following premature delivery. In addition, 10 blood samples from pregnant sheep were obtained for comparison with previous studies. The blood was collected in heparinized plastic syringes, centrifuged, and the plasma was stored at 4° until used. The COP of the plasma was measured on a 3-ml sample (large osmometer) or 0.3 ml sample (small osmometer) at room