expressed for H_f[5,6]. This expression takes the form of a polynomial with powers of H_f and is of the n + 1st order when n is the number of classes of binding sites. The value of the concentration of the free ligand H_f was obtained by finding the roots of this polynomial. The structure of the coefficients of this polynomial obeys simple permutation laws, and the coefficients can therefore be obtained by inspection of the powers of H_f. However, the expressions for the coefficients rapidly become very cumbersome for higher orders of the polynomial. An algebraic solution for the roots of the polynomial of higher than the 4th order is generally not possible. Therefore it is necessary to find these roots with iterative methods which is, even with the help of high speed digital computers, a time-consuming task.

Furthermore, different programs have to be coded for every number of classes of binding sites, since the coefficients of the corresponding polynomials are different for every order of the polynomial.

These disadvantages can be overcome by solving the binding equation implicitly as described above. The procedure LIGAND can be used without alterations for any possible number of classes of binding sites and is therefore very general. In addition, a solution of the binding equation to any specified possible degree of precision is obtained in much shorter time with the procedure described than with those which solve the binding equation by finding the roots of a polynomial.

The subroutine LIGAND is well suited for implementation in a scientific subroutine package and should be a valuable tool in studies where the total ligand concentration has to be corrected for unspecific binding[10].

Zusammenfassung. Es wird ein einfaches numerisches Verfahren beschrieben, mit dessen Hilfe die freien Konzentrationen eines Liganden, der an Blut- und Gewebebestandteile gebunden wird, berechnet werden können.

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Myocardial Inactivity of Therapeutic Concentrations of Hydralazine and Diazoxide

Hydralazine and diazoxide are effective vasodilator drugs used in the treatment of systemic hypertension[1-5]. They lower peripheral vascular resistance by a direct relaxing effect on arteriolar smooth muscle but have little action on capacitance vessels[1,3,5-7]. The hypotensive action of hydralazine and diazoxide is accompanied by marked increases in heart rate, left ventricular ejection rate, and cardiac output[1,2,4,5,8,9]. This cardiac hyperactivity is at least in part the result of increased sympathetic stimulation of the heart due to activation of the baroreceptor reflex by the vasodilation-induced hypotension[1,4,5,10-12]. It has been suggested that hydralazine and diazoxide may also exert more proximate positive chronotropic and inotropic effects on the heart itself[5,13,14]. They might produce such effects by releasing norepinephrine from myocardial sympathetic nerve endings, by stimulating cardiac β-adrenergic receptors, or by a direct myocardial action. We have recently established the serum concentrations of hydralazine[15] and diazoxide[3] that occur during the therapeutic use of these drugs in man. The present study was undertaken to determine if hydralazine or diazoxide exert any direct myocardial actions in such concentrations.

Materials and methods. Right ventricular papillary muscles and left atrial strips from kittens (0.4–0.9 kg) and right atrial pacemaker preparations from guinea-pigs (0.3–0.4 kg) were used. To insure adequate oxygenation of the central fibres of papillary muscles, only muscles of less than 0.6 mm² cross-sectional area were chosen[16]. The preparations were fixed between a small plastic electrode block and a stainless-steel wire hook

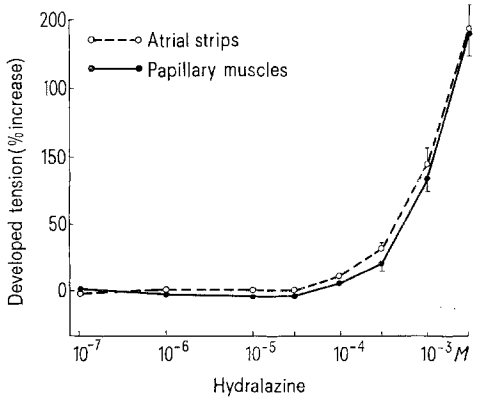

Fig. 1. Effect of hydralazine on isometric tension developed by 12 kitten papillary muscles and 12 kitten atrial strips. Means ± SEM.

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extending upward to a strain-gauge transducer. Lenght-tension curves were determined for individual papillary muscles and atrial strips, and each was maintained at one-half of the resting tension necessary for maximal tension development. For pacemaker preparations resting tensions just sufficient to record tension were used. Papillary muscles and left atrial strips were stimulated through 2 punctate platinum electrodes contacting the surface of the muscles just above the point of clamping. The preparations were driven 60 times per min by square-wave pulses of 5 msec duration. Stimulus intensity was just above threshold in order to prevent release of norepinephrine from adrenergic nerve endings. Isometric tension was measured with strain-gauge transducers and recorded on a direct-writing oscillograph. We have previously described details of the stimulation and recording devices[17,18].

All preparations were mounted in a 50 ml organ bath containing a physiologic salt solution[17,18]. The bath solution was continuously oxygenated and stirred by a gas mixture of 95% O_2 and 5% CO_2. After equilibration with this mixture the bath pH was 7.4. The temperature of the solution was always maintained at 37.5 $\pm$ 0.1 °C. The volume of solution of hydralazine hydrochloride, sodium diazoxide, and D,L-propranolol hydrochloride added never exceeded 0.1% of the organ bath volume. Concentration-effect curves were determined by cumulative increases of drug concentration in steps of 1/2 log unit. The next higher concentration was added as soon as the full effect of the previous concentration had been reached. Propranolol used (10^{-6} M) had no chronotropic or inotropic effect on the preparations, but strongly antagonizes the myocardial actions of norepinephrine released from cardiac stores[17].

Results. In concentrations up to 10^{-4} M, hydralazine had no inotropic effects on kitten papillary muscles or atrial strips (Figure 1). Concentrations of hydralazine above 10^{-4} M progressively increased tension development by both atrial and ventricular myocardium. The almost 3 fold increased in tension development caused by 3×10^{-3} M hydralazine was accompanied by a 23% decrease in the time to peak tension. In 10 papillary muscles and 8 left atrial strips exposed to 10^{-6} M propranolol, hydralazine had no positive inotropic effects in concentrations up to 10^{-3} M. The increases in tension development produced by 3×10^{-3} M hydralazine were decreased from 195% and 197% to 12% by 10^{-6} M propranolol. 5 papillary muscles from 3 kittens treated i.p. 24 h prior to the experiment with reserpine 1 mg/kg showed no inotropic response to hydralazine 3×10^{-3} M.

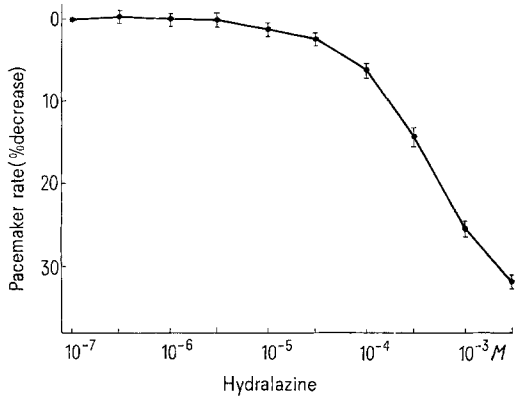

Fig. 2. Effect of hydralazine on guinea-pig pacemaker rate. Means $\pm$ SEM of 12 preparations.

No concentration of hydralazine increased the rate of impulse formation in guinea-pig pacemaker preparations (Figure 2). Concentrations above 10^{-5} M had progressive negative chronotropic effects.

Diazoxide in concentrations up to 10^{-4} M had neither positive nor negative inotropic or chronotropic effetcs on 8 kitten papillary muscles, 10 kitten left atrial strips, and 10 guinea-pigs right atrial pacemaker preparations. Higher concentrations could not be studied because of the relative insolubility of the drug.

Discussion. Serum concentrations of hydralazine during antihypertensive therapy range from 0.1 to 2.0 mg/l[15]. In therapeutic concentrations hydralazine is approximately 80% bound to human serum proteins[19]. Thus, the free serum concentration of hydralazine, which is in equilibrium with the extracellular fluid, ranges below 0.4 mg/l or 2×10^{-6} M. This concentration is two orders of magnitude below that which produces a positive inotropic effect. It is clear that therapeutic concentrations of hydralazine have no direct chronotropic or inotropic effects.

The significant positive inotropic actions of greatly supratherapeutic concentrations of hydralazine are associated with considerable shortening in the time to maximum tension development, as is seen during the action of norepinephrine[20]. They are strongly antagonized by propranolol and are absent in myocardium from which norepinephrine has been depleted by reserpine. Thus, they are almost certainly due to the release of norepinephrine from adrenergic nerve endings in the myocardium. The ability of hydralazine to release norepinephrine has been previously suggested by studies on the contractile force of guinea-pig atria[14]. Positive chronotropic effects due to norepinephrine release in pacemaker preparations are probably prevented by a direct negative chronotropic effect of high concentrations of hydralazine.

Serum concentrations of diazoxide after single therapeutic injections of 300 mg are about 20 mg/l, but they can rise as high as 100 mg/l after repeated injections[3]. Diazoxide is approximately 90% bound to serum albumin[3], so that a total serum concentration of 100 mg/l corresponds to 10 mg/l of the free drug (4×10^{-5} M). This concentration is less than half the highest concentration studied in these experiments. Accordingly, therapeutic concentrations of diazoxide have neither chronotropic nor inotropic actions on the myocardium.

Zusammenfassung. Therapeutische Konzentrationen von Hydralazin (10^{-7} -2×10^{-6} M) und von Diazoxid (5×10^{-6} -4×10^{-5} M) haben keine inotropischen oder chronotropischen Wirkungen auf isolierte Herzmuskeln von Katzen und Meerschweinchen. Hydralazin Konzentrationen über 10^{-4} M erhöhen die Spannungsentwicklung durch Freisetzung von endogenem Noradrenalin aber vermindern die Schrittmacherfunktion.

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