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Summary

A new synthesis of flavopereirine is described, starting from the quaternary salts of gramine and N-phenacyl-3-ethyl-pyridinium bromide.

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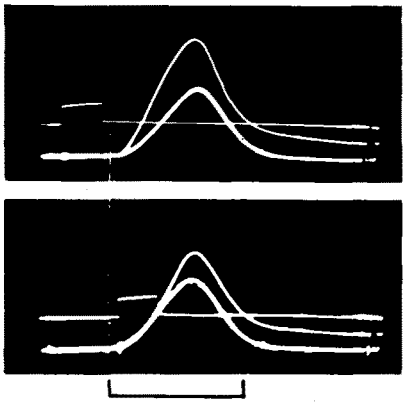
Effect of Increasing the Calcium Concentration During a Single Heart-Beat[1]

Ca ions serve as a link between electrical and mechanical activity[2–4]; their concentration is an important factor determining the contractile strength. The question arises whether it is the Ca level at the *onset* of electrical activity which sets the degree of shortening (trigger effect), or whether Ca ions are essential throughout the phase of electrical activity in order to link shortening to membrane depolarization. The plan of the experiment was to raise the Ca concentration rapidly, after the beginning of an action potential, and to see whether this would still result in a stronger contraction.



Effect of admixing Ca ions to the perfusate of a turtle ventricle. In each of the two records, the film was exposed during three successive contractions, of which the first two coincide. Calcium was admixed, as indicated by the 2·5 sec square pulse, preceding the third beat (top record) and during the third beat (bottom record). The retouched vertical line marks the moment of stimulation.

Turtle ventricles were immersed in a bath of cooled Ringer's solution (8°C) and driven electrically at a rate of 1 beat/min. A cannula was inserted into the single coronary artery and the vascular bed was perfused with Ringer's solution of a low Ca concentration (0·7 *mM*). A calcium-rich solution (100 *mM* $CaCl_2$ in Ringer's) was kept ready in a thin tube ending inside the tip of the arterial cannula. Relatively small volumes of this solution could be admixed for periods of 2·5 sec. Contractions were recorded by means of a mechano-electrical transducer (RCA 5734). The method has been described in more detail in connexion with a different type of experiment[5].

The Figure shows the effect of momentarily raising the Ca concentration before and during systole. The lower record makes it clear that an increment of contractile strength could still be obtained if the Ca concentration in the perfusate started to rise at the beginning of systole, i.e. after the onset of membrane depolarization.

Membrane potentials were recorded on several occasions by means of LING-GERARD[6] electrodes. A 'pulse' of calcium ions had the effect of slightly shortening the action potential. This would not, *per se*, be expected to result in a stronger contraction. It seems permissible, then, to conclude that Ca ions mediate between electrical and mechanical activity *throughout the period of membrane depolarization.*

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Zusammenfassung

An Schildkrötenherzen verstärkt eine Zugabe von Ca-Ionen, die *nach* Beginn des Aktionspotentials erfolgt, die bereits begonnene Kontraktion. Es wird daraus geschlossen, dass Ca-Ionen während der *ganzen* Erregungsphase (und nicht nur zu deren Beginn) zwischen der elektrischen und der mechanischen Aktivität vermitteln.

Calcium and the Activation of Contraction

It has recently been proposed[1] that the contraction of heart muscle is initiated by a negatively charged Ca-compound (an 'activator') which is moved during depolarization of the cell membrane from the surface into some deeper cellular compartment. This hypothesis is based for the most part on an analysis of two experimental observations on the frog's heart: (1) that tension developed in twitches and contractures depends on the ratio $[Ca]/[Na]^2$ (these being the Ca and Na concentrations in the outside fluid)[2,3]. This observation suggested in turn that Na and Ca ions compete for a site or molecule 'R' which becomes active only when it combines with Ca. (2) Contracture tension obtained at a given ratio $[Ca]/[Na]^2$, i.e. at a given concentration of the proposed 'activator CaR', is larger the greater the degree of membrane depolarization (which was varied experimentally by altering the external K-concentration)[1]. This effect suggests that the normal mem-

[1] Aided by the Swiss National Science Foundation, Grant No. 1138.
[2] I. DE B. DALY and A. J. CLARK, J. Physiol. *54*, 367 (1921).
[3] H.-C. LÜTTGAU and R. NIEDERGERKE, J. Physiol. *143*, 486 (1958).
[4] R. NIEDERGERKE, Exper. *15*, 128 (1959).
[5] S. WEIDMANN, J. Physiol. *132*, 157 (1956).
[6] G. LING and R. W. GERARD, J. cell. comp. Physiol. *34*, 383 (1949).

[1] H. C. LÜTTGAU and R. NIEDERGERKE, J. Physiol. *143*, 486 (1958).
[2] W. WILBRANDT and H. KOLLER, Helv. physiol. Acta *6*, 208 (1948).
[3] R. NIEDERGERKE and H. C. LÜTTGAU, Nature, Lond. *179*, 1066 (1957).

brane potential acts as a barrier to the entry of 'activator' molecules, and therefore that CaR carries a negative charge.

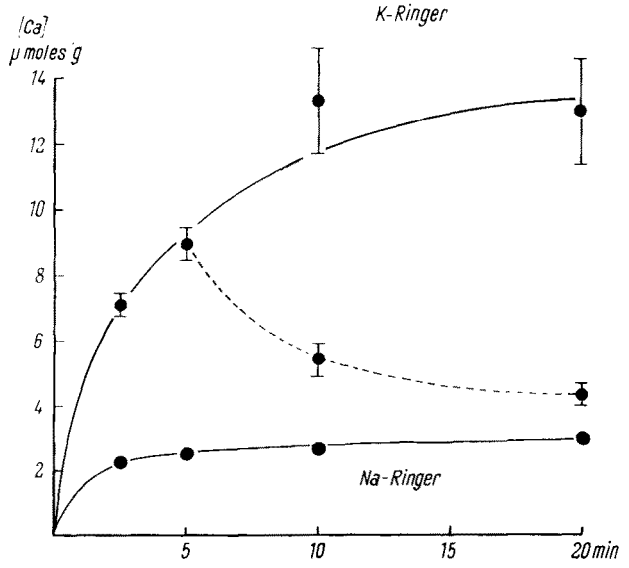

Fig. 1. – Increase of Ca-uptake by frog ventricles due to replacement of the Na in the outside fluid by K. Solid curves: amount of labelled Ca (in μ moles/g dry wt.*) taken up from fluids having either a normal NaCl concentration (Na-Ringer) or NaCl replaced by KCl (K-Ringer). Both fluids contained 1 *mM* labelled Ca. Dashed curve: ventricles exposed to Na-Ringer after a 5 min period in K-Ringer. The labelled Ca (1 *mM*) in the two fluids had identical specific activity. Each point of the upper curves is the mean of determinations with 5 different ventricles. Vertical bars: 2 × s.e. about the mean. (Ca-uptake with Na-Ringer is based on many more determinations than that with any other fluid, and its error is therefore correspondingly smaller.)

* Tissue was dried to constant weight at 80 °C.

Independent support for the existence of such a mechanism has been obtained by studying the uptake of ^{45}Ca by heart ventricles at rest and during contracture. Two series of experiments relating, respectively, to the two above-mentioned observations have been made. In the first, competition between Na and Ca was tested by perfusing ventricles with Na-depleted fluids. On the above hypothesis this should result in an increased cellular concentration of CaR and thus lead to an increase in ^{45}Ca-uptake. That Ca is taken up under such conditions has already been inferred from experiments in which it was found that heart strips accumulated ^{89}Sr when immersed in fluids in which NaCl had been replaced by either sucrose, choline Cl or LiCl[4]. This result has now been confirmed in more quantitative experiments using whole heart ventricles perfused with fluids containing ^{45}Ca. Furthermore, it has been found that Ca is taken up, to about the same extent, when the external Na was replaced by K. In Figure 1 the difference between the two solid lines illustrates this additional uptake of labelled Ca due to the total replacement of external Na by K. To find out how firmly the accumulated Ca was bound, some ventricles (dashed line) were transferred, after 5 min perfusion with KCl Ringer, into normal Ringer; both fluids contained labelled Ca of the same specific activity. The observation that ^{45}Ca is lost from the tissue under these conditions, despite a constant external concentration of labelled Ca, indicates that the cellular Ca concentration was raised during the period of Na deficiency. This supports the concept of a reversible competition between Na and Ca for a cellular site as invoked by the initial hypothesis. The additional Ca-uptake was associated with a strong contracture, while relaxation occurred early during the Ca-loss.

[4] R. Niedergerke and E. J. Harris, Nature, Lond. *179*, 1068 (1957).

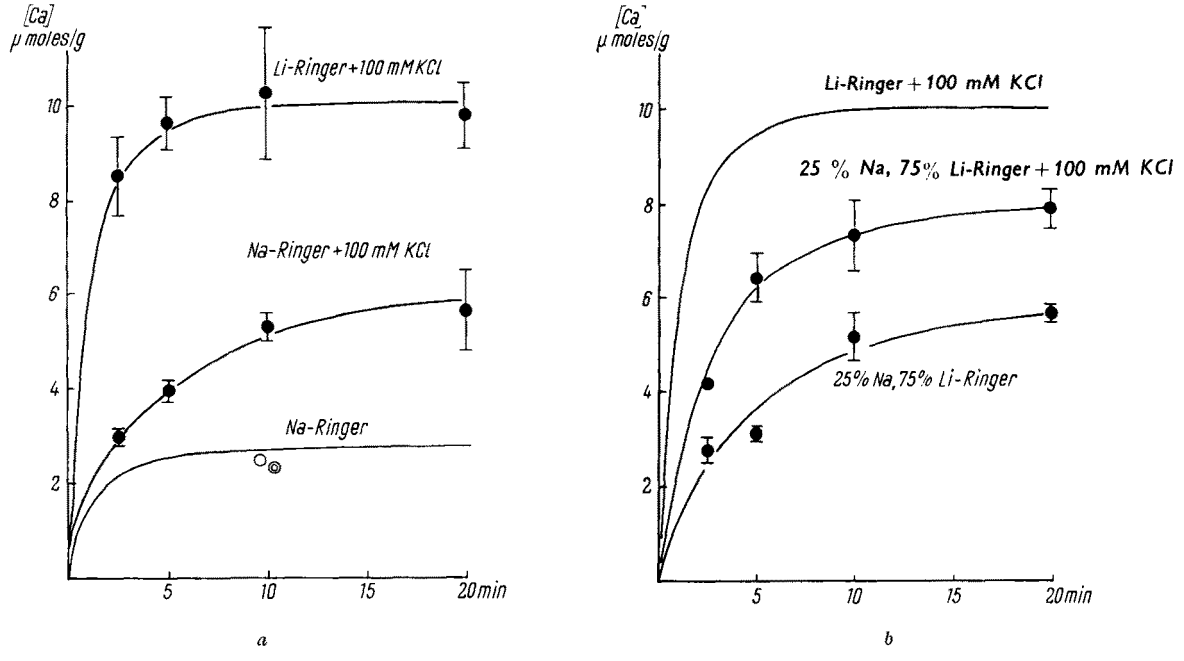

Fig. 2*a* and *b*. Effect of depolarization on the Ca-uptake of ventricles. The difference between lower two curves in each illustration shows additional Ca-uptake due 100 *mM* KCl either with Na-Ringer (Fig. 2*a*) or with 25% Na, 75% Li-Ringer (Fig. 2*b*) as perfusion fluid. Upper curve in each illustration shows maximal Ca-uptake in the absence of Na (NaCl replaced by LiCl) but with additional 100 *mM* KCl. Also plotted are two controls showing the Ca-uptake in hypertonic solutions (Fig. 2*a*): in each case a 10 min perfusion period was used with Na-Ringer containing either added 180 *mM* sucrose (hollow circle), or added 100 *mM* LiCl (double circle).

The aim of the second experiment was to test whether depolarization facilitates the entry into the cell of the postulated Ca compound; such evidence would be obtained if, at a constant value of external [Ca]/[Na]², depolarization were also to give rise to an uptake of labelled Ca. Ventricles were depolarized by raising the external K concentration to 100 *mM*; but this was done, in contrast to the first experiment, by adding KCl (solid) to the perfusion fluid. In this way the ratio [Ca]/[Na]² was kept constant. Figure 2 shows the effect of adding 100 *mM* KCl to (1) normal Ringer's fluid (Fig. 2*a*) and (2) to a fluid in which 75% NaCl had been replaced by LiCl (Fig. 2*b*), both fluids containing 1 *mM* labelled Ca. The two lower curves, in each figure, indicate that depolarization by added K does, in fact, increase the Ca-uptake. In the absence of Na (Li-Ringer + 100 *mM* KCl, see upper curves in both figures), a maximal Ca-uptake occurs which is comparable in magnitude to that obtained in Na-free Ringer without additional K, again in agreement with the initial hypothesis. Controls in which either 100 *mM* LiCl or 180 mM sucrose were added to Ringer's fluid (Fig. 2*a*) show that neither the increased ionic concentration nor the hypertonicity of the fluids are responsible for, or contribute to, the effects shown in Figure 2.

If, as these experiments suggest, inward movement of Ca activates the contraction of the heart, an increased Ca-uptake should also occur in the course of a single twitch. Evidence for this may be found in the fact that periods of electrical stimulation also increase uptake of labelled Ca (NIEDERGERKE, unpublished observation; cf. for similar effects in nerve[5] and skeletal muscle[6]). In this context WEIDMANN's recent observation[7] is of interest, viz. that the twitch tension of the heart can be enhanced by applying Ca momentarily during the course of a single action potential, which is also explicable by the initial hypothesis.

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Zusammenfassung

Froschherzventrikel nehmen eine zusätzliche Menge Calcium (bestimmt mit dem Isotop ^{45}Ca) aus der Perfusionsflüssigkeit auf:

1. wenn diese Na-frei ist (zum Beispiel, wenn NaCl ersetzt ist durch LiCl oder KCl);

2. wenn dieser zusätzliches KCl zugegeben und damit die erregbare Membran bei konstanter Na-Konzentration depolarisiert wird.

Gleichzeitig mit der erhöhten Ca-Aufnahme wird in beiden Fällen eine Kontraktur beobachtet. Das Ergebnis unterstützt die früher vorgeschlagene Hypothese, nach welcher die Kontraktion des Herzens durch eine spezifische Ca-Verbindung (einen «Aktivator») eingeleitet wird.

[5] A. L. HODGKIN and R. D. KEYNES, J. Physiol. *138*, 253 (1957).
[6] C. P. BIANCHI, in SHANES, Pharmacol. Reviews *10*, 252 (1958).
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Fibrinolyse im Kammerwasser menschlicher und tierischer Augen

Fibrinolytische Fermente sind in verschiedenen Körperflüssigkeiten und Organen nachgewiesen worden[1]. Meist handelt es sich dabei um Profermente, die erst durch Kinasen, Aktivatoren oder durch bestimmte chemische Behandlung in die aktive Form übergeführt werden. Um so überraschender war für uns die Feststellung, dass im Kammerwasser menschlicher und verschiedener tierischer Augen offenbar normalerweise eine geringe Spontanfibrinolyse nachweisbar ist.

Experimentelles. Die Kammerwasser wurden durch Punktion der vorderen Augenkammer in Lokalanästhesie beim Menschen, in Pentobarbitalnarkose bei Kaninchen und Katzen und unmittelbar nach dem Tode bei Pferden, Rindern und Schweinen gewonnen und bis zur Untersuchung eingefroren. Die fibrinolytische Aktivität wurde nach der von DAUSSET *et al.*[2] beschriebenen, indirekten, aber sehr empfindlichen Methode der passiven Hämagglutination nachgewiesen. Das Prinzip der Methode beruht darauf, dass beim Fibrinabbau durch Plasmin eine Substanz entsteht, die tannierte und mit Fibrinogen behandelte Erythrozyten agglutiniert. Je stärker die Fibrinolyse, um so höher der Agglutinationstiter.

In Fibrinolyseansätzen ohne Streptokinase (SK) wurde die aktuelle, in Ansätzen mit SK die durch diese Aktivierungsart maximal erreichbare Fibrinolyse bestimmt. Die Ansätze enthielten folgende Komponenten:

0,1 ml Kammerwasser;
0,2 ml Rinder-Citratplasma als Fibrinogenquelle;
0,2 ml physiolog. NaCl oder 200 E SK (Varidase);
0,1 ml (4 E) Thrombin (Topostasin Roche).
Inkubation während 30 min bei 37°C.

Anschliessend wurde vom eventuell noch vorhandenen Gerinnsel abzentrifugiert und zur Agglutination genau nach der Vorschrift von DAUSSET *et al.*[2] verfahren.

Ergebnisse. Die Agglutinationstiter, die ein Mass für die Fibrinolysestärke sind, finden sich in folgender Tabelle:

Spezies	*n*	Agglutinationstiter	
		Ohne SK	Mit SK
Mensch .	8	1:4 bis 1:10	1:4000 bis 1:8000
Katze . .	5	1:10 bis 1:20	1:100 bis 1:400
Kaninchen	5	1:2 bis 1:8	1:40 bis 1:200
Schwein .	2	1:1 bis 1:2	1:10
Pferd . .	3	1:10 bis 1:20	1:10 bis 1:40
Rind . .	3	1:2 bis 1:10	1:2 bis 1:10

Wir dürfen annehmen, dass die durchwegs niedrigen Agglutinationstiter in den SK-freien Ansätzen tatsächlich einer geringen Spontanfibrinolyse entsprechen, da nicht geronnene, entweder fibrinogen- oder thrombinfreie Kontrollen negativ blieben. Analoge Ansätze mit Plasma anstelle des Kammerwassers sind höchstens unverdünnt schwach positiv. In der Tabelle sind die Tierarten in der Reihenfolge der SK-Aktivierbarkeit des fibrinolytischen Systems ihres Kammerwassers bzw. dessen Proaktivatorgehaltes[3] angeordnet. Die maximale SK-Aktivierbarkeit des Kammerwassers bleibt hinter derjenigen des Plasmas zurück, beträgt doch der Agglutinationstiter im analogen Plasmaansatz für den Menschen 1:10000 bis 1:20000, für Katzen und Kaninchen 1:100 bis 1:1000. Die SK-aktivierten Kammerwasser von Mensch und Katze lysieren

[1] Lit. bei E. DEUTSCH, *Blutgerinnungsfaktoren* (F. Deuticke, Wien 1955).
[2] J. DAUSSET, Y. BERGEROT-BLONDEL und M. COLIN, Vox sanguinis *1*, 95 (1956).
[3] ST. MÜLLERTZ, *Thrombose und Embolie* (Benno Schwabe, Basel 1955), p. 75.