water as the solvent: Diluent A 8.5 g NaCl, 26.7 g sucrose, 0.7 g glucose, 0.4 g fructose; Diluent B 8.5 g NaCl, 26.7 g sucrose, 0.4 g fructose; Diluent C 8.5 g NaCl, 26.7 g sucrose, 0.7 g glucose; Diluent D 8.5 g NaCl, 0.7 g glucose, 0.4 g fructose; Saline 8.5 g NaCl.

Five replicates of the following step-wise procedure were run for each of the saline and sugar diluents: A, B, C, and D. 4 test tubes, hereafter noted as 1, 2, 3, and 4, were used in each replicate. Tubes 1 and 2 each received 1 ml of saline diluent; tubes 3 and 4 each received 1 ml of the saline and sugar diluent being tested.

Step A: 1 µl of honey bee semen introduced into each tube and gently mixed with the diluent. Step B: 1 drop of the mixture from each tube placed on a separate microscope slide, covered with a coverslip, and spermatozoal motility and morphology appraised by phase contrast microscopy. Step C: Motility of spermatozoa in tube 1 rechecked, and then 3 drops (approx. 0.16 ml) of the test saline and sugar diluent added to it. Motility checked again after this addition. (Final approximate sugar concentrations here and at Step E below were 0.39% for diluents A, B, and C and 0.016% for diluent D.) Step D: Motility in tube 2 rechecked and then oxygen bubbled through the mixture for 1 min followed by another check for motility. Step E: 3 drops of the test saline and sugar diluent added to oxygenated tube 2 and again checked for motility. Step F: Final reexamination of tubes 3 and 4 to verify observation in Step B.

In addition, a series of on-slide motility examinations was made for each saline and sugar diluent, using saline as a control, as follows: 1 drop of saline was placed on each of 2 slides. 1 drop of saline and sugar diluent was placed on a third slide. Then 1 drop of semen was placed into the fluid on each slide, covered with a coverslip, and examined microscopically for spermatozoal motility. Next a drop of saline was placed adjoining the coverslip on one of the saline slides, and a drop of saline and sugar diluent was placed likewise on the other saline slide. All 3 slides were again examined microscopically.

Results and discussion. In both series of tests, differences in spermatozoal motility between saline-semen mixtures and saline and sugar-semen mixtures were so great that quantification in terms of percent motile spermatozoa was unnecessary. The response was nearly all-or-none.

Few spermatozoa (not more than 1–2%) were capable of movement in the saline-semen mixtures. Nearly all were folded at the center of their length. In comparison, nearly all spermatozoa in all of the saline and sugar-semen mixtures exhibited vigorous, normal-appearing motility and normal morphology. No differences were detected in relation to the omitted sugars in diluents B, C and D.

Oxygenation of the semen-saline mixtures did not induce motility and did not seem to alter the folded configuration of the spermatozoa. In contrast, adding 3 drops of any saline and sugar diluent to either oxygenated or unoxygenated saline-semen mixtures immediately produced vigorous movement of the spermatozoa although most remained folded.

The results of the on-slide motility tests were consistent with the foregoing. Semen placed in saline remained in a tight clump, and nearly all of the spermatozoa were immotile. Semen placed in any of the saline and sugar diluents immediately dispersed into swirling foci of motile spermatozoa. Additional saline allowed to diffuse into the original semen-saline preparations had no effect other than partial disruption of the clump by streaming diluent. In contrast, swirling foci of motile spermatozoa formed rapidly when any of the saline and sugar diluents were allowed to diffuse into the semen-saline preparations.

These results suggest that sugars in concentrations as low as 0.016% can induce spermatozoal motility in dilute mixtures of honey bee semen and 0.85% saline. They also indicate that no one of the sugars tested is indispensable for the induction and support of spermatozoal motility. We suppose that motility occurred in mixtures containing sugar because the sugar provided a metabolizable energy source not previously available. This conjecture is supported by similar findings in other species[1] and by observation of rapid fructolysis in honey bee semen[2].

The motility difference between saline and sugar-semen mixtures and saline-semen mixtures of equivalent dilution is evidence that sugar was more important to spermatozoal motility than the dilution itself. This is inconsistent with the conclusion of others[3,5] that motility is induced merely by dilution, probably with secretions of the spermathecal gland, when spermatozoa are released or removed from the spermatheca. Further work should be done on the composition of spermathecal gland secretion to determine whether it too contains significant sugar.

Zusammenfassung. Es wird gezeigt, dass frischgewonnenes Sperma der Honigbiene *Apis mellifera* L. in 0,85% NaCl-Lösungen mit Rohr-, Frucht- und Traubenzuckerzusatz bewegungsfähig bleibt, während in 0,85% NaCl nur wenige Spermien bewegungsfähig sind.

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Interrelationships Between the Contractile Effects of Metabolic Substrates, β-Adrenergic Blocking Agents and Endogenous Catecholamine Depletion on Isolated Rat Atria[1]

The effects of adrenergic influences on the contraction of the heart muscle have been extensively studied over the years. On the other hand we have explored[1–8] the importance of metabolic substrates and enzyme inhibitors to maintain and modify electrical and contractile characteristics of the isolated myocardium. However, little is known regarding the possible mutal influences between these two aspects.

In the present study we have attempted to explore whether the myocardial contractile effects elicited by

catecholamine depletion or adrenergic blocking agents could be altered by the presence of different metabolic substrates. Also, the influences of catecholamine depletion and adrenergic blocking agents upon the myocardial effects of substrates were studied.

Methods. Female adult rats were decapitated, the atria removed, suspended in a modified Krebs-Ringer-Bicarbonate medium (KRB) at pH 7.4 and 30 °C, and stimulated at a rate of 200/min with twice the threshold intensity, as previously reported[1-5]. The substrate for the medium was glucose or pyruvate at 5.5 mM. A 60 min equilibration period was allowed before readings of the isometric developed tension, recorded by means of a strain gauge, were taken. The recorded developed tension at the end of the equilibration period is referred to in the text as the initial developed tension (IDT).

In one group of experiments, the contractile effects of $10^{-5}M$ DL-propranolol or $10^{-3}M$ DL-MJ 1999 were tested by additions performed at the end of the equilibration period. (Unless otherwise stated the concentration of drugs indicated in the text refer to the final concentration in the tissue bath.) In order to test the adequacy, as well as to compare the degree of the β-adrenergic blocking action, the tissue was exposed to 0.01 µg/ml isoproterenol or to 0.2 µg/ml norepinephrine, before and after the addition of the blockers. Only those atria on which the β-blockers reduced by 100% or 50% the stimulation of developed tension elicited by isoproterenol or norepinephrine respectively, were considered for experimental analysis.

In another group of experiments the contractile effects of 11.0 mM pyruvate were tested on normal atria equilibrated in KRB medium with 5.5 mM glucose, both in the absence or in the presence of $10^{-5}M$ DL-propranolol or $10^{-3}M$ DL-MJ 1999 added to the bath 30 min prior to the addition of pyruvate.

A final group of experiments were performed on atria isolated from normal or reserpinized animals receiving i.p. injections of 5 mg/kg reserpine 24 h prior to the sacrifice. Atrial depletion of catecholamines was complemented in all cases by the in vitro addition of tyramine (7 µg/ml) up to the point of absence of response.

All the developed tension values were recorded in mg, and experimental variations, expressed as percentage changes under different experimental conditions, were compared using the Student's t-test. Differences were considered significant if p was 0.05 or less.

Results. The stability of developed tension (SDT) of control and catecholamine depleted atria was studied in preparations suspended in KRB medium with 5.5 mM glucose or pyruvate, by determining the spontaneous changes of the IDT over a period of 60 min. SDT was similar in both groups, a 5–6% spontaneous decrement of contractile tension being observed at the end of 60 min.

Figure 1 shows that the IDT of catecholamine depleted rat atria suspended in 5.5 mM glucose was comparable to that of controls, the result being in agreement with that of TANZ and MARCUS[6], on cat papillary muscle. However, as can be seen in Figure 1, the IDT, of catecholamine depleted atria suspended in a medium with 5.5 mM pyruvate was greater than in all the other cases.

The contractile effects of a concentration of DL-propranolol or DL-MJ 1999, that produced a comparable blockade of the stimulation of the developed tension elicited by isoproterenol or norepinephrine were explored on control rat atria suspended in KRB medium with 5.5 mM glucose or pyruvate as the substrate. The Table shows that the decrement of developed tension induced by $10^{-5}M$ DL-propranolol is significantly greater when the substrate was glucose than when it was pyruvate. On the other hand, the small but consistent depression of the developed tension observed after $10^{-3}M$ DL-MJ 1999 to atria suspended in KRB medium with 5.5 mM glucose, is no longer present when the substrate is pyruvate.

As can be seen in Figures 2 and 3, curves I, the addition of 11.0 mM pyruvate to normal control atria suspended in KRB medium with 5.5 mM glucose, produced initially a transient and marked depression of the IDT, followed by a late and partial recovery; the levels of developed tension 1 h after the addition being significantly smaller than those of untreated atria (percentage change of IDT 60 min after pyruvate: -16 ± 1.8, $n = 7$). This effect of pyruvate, which is not merely dependent on

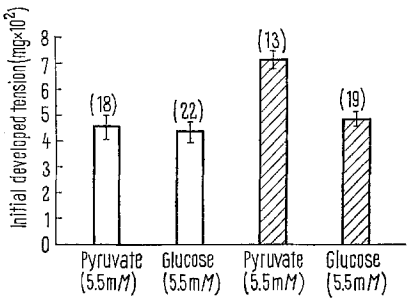

Fig. 1. Initial developed tension of normal and catecholamine depleted rat atria suspended in different substrates. White columns, IDT mean values of normal rat atria; shaded columns, IDT mean values of catecholamine depleted rat atria by a combination of in vivo reserpinization and in vitro tyraminization. Vertical bars, SEM. Numbers in parentheses refer to the number of atria.

Effect of DL-propranolol and DL-MJ-1999 on the developed tension of isolated rat atria suspended in different substrates[a]

Substrate	DL-Propranolol ($10^{-5}M$)	DL-MJ-1999 ($10^{-3}M$)
	% Change of developed tension[b]	
Glucose (5.5 mM)	(A) -29.5 ± 2.3 ($n = 6$)	(C) -9.2 ± 2.6 ($n = 7$)
Pyruvate (5.5 mM)	(B) -12.9 ± 4.5 ($n = 6$)	(D) -0.7 ± 4.0 ($n = 10$)

(A) vs (B) $p < 0.01$ (C) vs (D) $p < 0.05$

[a] The concentrations of DL-propranolol and DL-MJ-1999 employed produced a 50% blockade of the stimulation of developed tension elicited by norepinephrine and a 100% blockade of the stimulation of developed tension elicited by isoproterenol. [b] The percentage changes of developed tension reported are those observed at the end of a 90 min period after the addition of the drugs. Mean ± S.E.M.

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the accompanying Na^+ ions of the molecule, nor it is related to changes in the osmolarity of the medium or to cholinergic mechanisms[1], is however clearly altered by adrenergic β-blockers as well as by the depletion of atrial endogenous catecholamines by reserpine and tyramine. Indeed, Figure 2, curve 3, shows that the pattern of atrial contractile activity after pyruvate is modified in the catecholamine depleted group, the late depression of developed tension being significantly smaller than that of the normal controls (percentage change of IDT 60 min after pyruvate: -0.59 ± 4.2, $n = 8$; catecholamine depleted vs controls, $p < 0.01$). Also, as shown in Figures 2 and 3, curve 2, a modification of the late contractile influence induced by 11.0 mM pyruvate was observed on atria incubated with $10^{-5}M$ DL-propranolol (percentage change of IDT 60 min after pyruvate: $+6.9 \pm 3.6$, $n = 7$; DL-propranolol treated vs controls, $p < 0.001$) or with $10^{-3}M$ DL-MJ 1999 (percentage change of IDT 60 min after pyruvate: -6.0 ± 3.2, $n = 7$; DL-MJ 1999 treated vs controls, $p < 0.01$). On the contrary, the presence of these adrenergic β-blockers, or the depletion of endogenous catecholamines by reserpine and tyramine were unable to modify significantly the early transient depression of atrial IDT observed after the addition of 11.0 mM pyruvate.

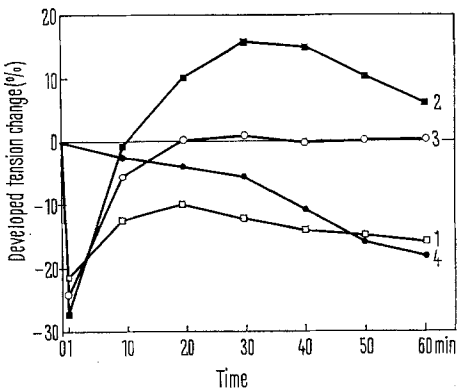

Fig. 2. Effect of pyruvate on the developed tension of normal, catecholamine depleted and DL-propranolol treated rat atria. Atria were equilibrated in 5.5 mM glucose medium and the IDT recorded. 11.0 mM pyruvate was added inmediately after (0 time). The effect of $10^{-5}M$ propranolol on a control series of experiments was also tested. Each point represents the mean of 6 to 8 experiments. Curve 1: Pyruvate on 7 normal atria with no extra additions. Curve 2: Pyruvate on 7 atria incubated in $10^{-5}M$ DL-propranolol. Curve 3: Pyruvate on 8 catecholamine depleted atria by reserpinization and tyraminization. Curve 4: DL-propranolol $10^{-5}M$ on 6 normal atria.

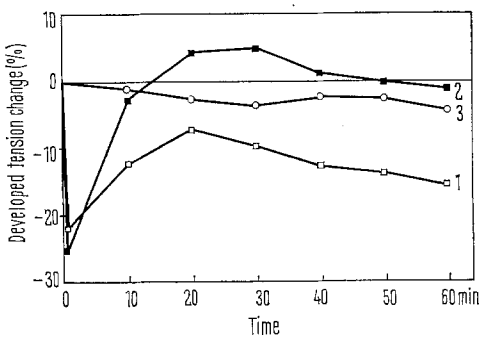

Fig. 3. Effect of pyruvate on the developed tension of normal and DL-MJ-1999 treated rat atria. Conditions as described in Figure 1. The effect of $10^{-3}M$ DL-MJ-1999 on a control series of experiments was also tested. Curve 1: Pyruvate on 7 normal atria with no extra additions. Curve 2: Pyruvate on 10 atria incubated in $10^{-3}M$ DL-MJ-1999. Curve 3: DL-MJ-1999 on 7 normal atria.

Discussion. The results of this investigation indicate that there are evident interrelationships between atrial contractile effects of metabolic substrates, β-adrenergic blocking agents and depletion of endogenous catecholamines.

The experimental evidence also indicate that the effect of DL-propranolol and DL-MJ 1999 on the developed tension of isolated rat atria cannot be simply explained by the blockade of β-receptors. This finding is in agreement with that of LEVY and RICHARDS[7], and LEVY[8], on isolated rabbit atria.

We have also shown that the reduction of developed tension induced by the addition of DL-propranolol or DL-MJ 1999 to atria suspended in KRB medium with glucose as the substrate, was significantly greater than that observed with pyruvate as the substrate. Furthermore, the IDT of catecholamine depleted atria suspended in KRB medium with pyruvate, was greater than that in glucose as well as than that of control atria suspended in glucose or pyruvate. Therefore, it is evident that after catecholamine depletion, or in the presence of β-adrenergic blockers, pyruvate became a better substrate than glucose for the maintenance of higher levels of the developed tension of isolated rat atria.

It is evident from the present results that catecholamine depletion by a combination of reserpinization-tyraminization, as well as the presence of adrenergic β-blockers, can significantly alter the late phase of the depressive influence of high concentrations of pyruvate on the contractile tension of isolated rat atria. However, as the same treatments did not alter the early transient depression of developed tension induced by pyruvate, it would appear that the effects of pyruvate on atrial contraction are complex and possibly associated to different mechanisms. As previously described[1-3,5] the developed tension of isolated rat atria is particularly sensitive to the presence of exogenous glucose and to the integrity and normal operation of the Embden-Meyerhof pathway. Also it was postulated, based on biochemical findings[9-14], that the depression of atrial contractile tension observed after the addition of increasing concentrations of pyruvate or after treatments able to induce alterations on the rate of utilization of pyruvate via the tricarboxylate cycle, could be a consequence of the inhibition of the metabolism of glucose[1,5].

Although the present experimental evidence does not permit a definitive conclusion regarding the possible mechanism(s) responsible for the modifying effect that catecholamine depletion or adrenergic β-blockers have

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on the contractile influences of pyruvate, we suggest, as a working hypothesis, that such action could be associated with a faster rate of the metabolism of pyruvate, a substrate which, when accumulated readily, depresses the developed tension of isolated rat atria[1,3]. Indeed, the effects of catecholamine depletion, β-adrenergic blocking agents and catecholamines on the myocardial metabolism of fatty acid[15,16] could result in changes of the tissue levels of A-CoA, a metabolite known as a controller of the activity of pyruvate dehydrogenase[17] and thereby as a regulator of the rate of entry of pyruvate in the tricarboxylate cycle[18,19].

Résumé. La réduction de l'amplitude de la tension contractile auriculaire produite par le DL-propanolol ou par le MJ 1999 ne peut être considérée uniquement comme une conséquence du blocage des récepteurs β-adrénergiques. D'autre part, l'action inotropique des drogues citées est nettement différente suivant que les oreillettes se trouvent dans un milieu contenant de la glucose ou du pyruvate. La dépression contractile provoquée par l'agrégat du pyruvate fut nettement moindre dans les oreillettes déplétionées de cathécholamines ou en présence de DL-propanolol ou du MJ 1999.

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On the Mechanism of Amphetamine Potentiation by Iprindole

Recent studies with tyrosine hydroxylase inhibitors have suggested that amphetamine is an indirectly acting sympathomimetic amine whose central action depends upon the uninterrupted synthesis of catecholamines[1-3]. Desipramine (DMI) and other tricyclic antidepressants which block the uptake mechanism for norepinephrine in central norepinephrine fibers[4,5] have been shown to enhance and to prolong the central effects elicited by amphetamine in the rat[6-9]. The enhancement and the prolongation of the action of amphetamine after the administration of DMI-like antidepressants appears to be the consequence of an inhibition of the metabolism of amphetamine[10-14]. Iprindole, a new tricyclic antidepressant, does not appear to block the uptake of norepinephrine through the neuronal membrane but nevertheless strikingly enhances many central effects of amphetamine[15]. The present studies were undertaken to determine whether these actions of iprindole might also be the consequence of a modification of the distribution or metabolism of amphetamine.

Male Sprague-Dawley rats (180–220 g) were used. Amphetamine was administered i.v. as the sulfate salt and iprindole was injected i.p. as the hydrochloride 30 min before the administration of amphetamine. D-Amphetamine-H^3-sulphate (generally labeled, 4.23 c/mmol) was obtained from the New England Nuclear Corporation. The drug was assayed by a modification of the method of AXELROD[16] as previously described[10]. Psychomotor stimulation was measured in Williamson activity cages over a period of 10 h.

A single dose of iprindole enhances and strikingly prolongs the psychomotor activity elicited by D-amphetamine (Figure 1). For example, in rats pretreated with iprindole (2 mg/kg), D-amphetamine (2 mg/kg) elicited a marked psychomotor stimulation as long as 9 h after its administration. In animals which had not been pretreated with iprindole the action of the same dose of amphetamine persisted for only 2 h, with the maximum activity occurring at 1 h. Iprindole given alone did not evoke a measurable stimulation. It is noteworthy that a five-fold increase in the dose of iprindole did not increase the potentiation and prolongation of the action of amphetamine.

The measurement of H^3-D-amphetamine in brain revealed that the levels of D-amphetamine decreased logarithmically (Figure 2) in both control and iprindole pretreated animals. In animals pretreated with iprindole, the brain levels of D-amphetamine were not only higher than those of control animals but declined at a slower rate ($t\text{-}^1/_2$ for amphetamine approximately 1 h, $t\text{-}^1/_2$ for iprindole and amphetamine approximately 4 h). The analysis of the homogenates of the bodies of these animals showed that pretreatment with iprindole not only resulted

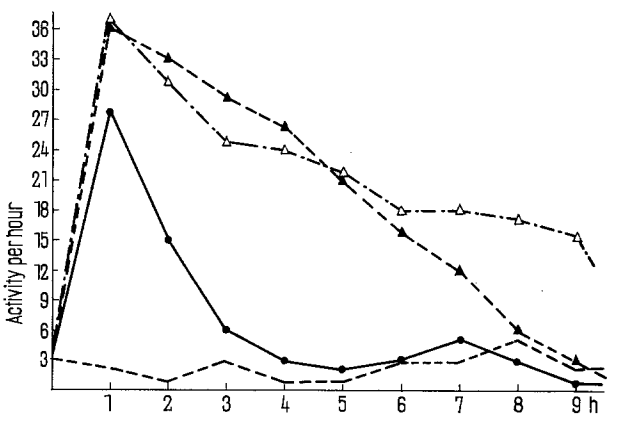

Fig. 1. Effect of iprindole on psychomotor stimulation elicited by D-amphetamine. ●—●, D-amphetamine; △—△, iprindole (2 mg/kg) + D-amphetamine; ▲—▲, iprindole (10 mg/kg) + D-amphetamine; ---, iprindole (10 mg/kg). Psychomotor activity is expressed as integrated counts per hour. Each value represents the mean of 6–10 animals. Iprindole was given i.p. 30 min before D-amphetamine (2 mg/kg per i.p.).