The Effects of Oxamate on the Contractility of Isolated Rat Atria

Oxamate, $^{+}H_3N$–CO–COO^{-}, a compound structurally related to pyruvate, is an inhibitor of lactate dehydrogenase from heart[1] and skeletal muscle[2], the inhibition being competitive with respect to pyruvate. Oxamate depressed anaerobic glycolysis and aerobic glucose utilization in tumors and human leucocytes, but endogenous respiration is unaffected[2–4]. There is evidence that glucose uptake or utilization through the Embden-Meyerhof pathway is important for a fraction of atrial contractility[5–7], and thus the effects of oxamate of atrial function were studied.

Methods. Rat atria were suspended in a modified Krebs-Ringer bicarbonate medium and electrically stimulated at a rate of 200/min. Developed tension, resting and action potentials were recorded as previously described[5,6,8].

Results. The effects of oxamate on atria in normal glucose medium are illustrated in Figure 1 (curves 1–3). The contractility falls fairly rapidly over the first 10 min and then remains at this depressed level with little change for at least 90 min. The addition of 2.75–5.5 m*M* pyruvate or lactate 30 min after oxamate does not effectively counteract the atrial depression (curves 4–7); indeed, pyruvate at 5–11 m*M* further reduces the contractility (curves 7 and 8) slightly more than in the absence of oxamate (curves 9 and 10). These effects are not due to increased osmolarity or Na^{+} concentration, since 11 m*M* excess NaCl affects contractility very little (curve 11) – the developed tension of untreated atria falls 5–10% over 90 min – and does not alter the effects of oxamate (curve 12). Inasmuch as the depression was identical with the inhibitor alone or in combination with 0.01 m*M* atropine, cholinergic mechanisms do not appear to be involved. On the other hand it is interesting to note that the initial rapid fall of contractility during the first 10 min after oxamate was accompanied by a transient 15% reduction in the rate of depolarization of atrial cells, with no other consistent changes in resting or action potentials.

The simultaneous addition at 0 time of 5.5 m*M* lactate (Figure 2, curve 2) or 2.75 m*M* pyruvate (Figure 2, curve 3) with 10 m*M* oxamate, however, protects the atria against the depression by oxamate alone (Figure 2, curve 1). Higher concentrations of lactate and pyruvate are ineffective (Figure 2, curves 4–6).

Atrial contractility is depressed in the absence of glucose (Figure 3, curve 1) or by 10 m*M* 2-deoxyglucose (Figure 3, curve 2); oxamate increases the depression in both cases (Figure 3, curves 3 and 4). Pyruvate 11 m*M* partially restores the contractility in the absence of glucose, with or without oxamate (Figure 3, curves 5 and 7), but has no protective effect in glucose medium in the presence of oxamate with or without 2-deoxyglucose (Figure 3, curve 6 and Figure 1, curve 8).

Discussion. Oxamate depresses atrial contractility in the presence or absence of glucose, and when added with 2-deoxyglucose or atropine but the results do not answer the question as to its mechanism(s) of action. They do provide, however, some preliminary data which open interesting possibilities regarding the metabolic control of atrial function. Can the action of oxamate be explained on the basis of a blockade of the conversion of pyruvate

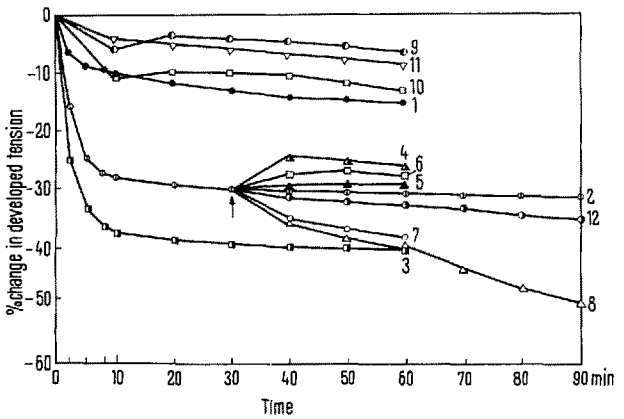

Fig. 1. Effects of oxamate on normal atria and of lactate, pyruvate and excess NaCl on atria depressed by oxamate. Curve 1, oxamate 5 m*M* at 0 time (4 atria); curve 2, oxamate 10 m*M* at 0 time (9 atria); curve 3, oxamate 20 m*M* at 0 time (9 atria); curve 4, oxamate 10 m*M* at 0 time, 2.75 m*M* lactate at 30 min (6 atria); curve 5, oxamate 10 m*M* at 0 time, 5.5 m*M* lactate at 30 min (6 atria); curve 6, oxamate 10 m*M* at 0 time, 2.75 m*M* pyruvate at 30 min (6 atria); curve 7, oxamate 10 m*M* at 0 time, 5.5 m*M* pyruvate at 30 min (7 atria); curve 8, oxamate 10 m*M* at 0 time, 11 pyruvate at 30 min (9 atria); curve 9, pyruvate 5.5 m*M* at 0 time (12 atria); curve 10, pyruvate 11 m*M* at 0 time (9 atria); curve 11, NaCl 11 m*M* excess at 0 time (6 atria); curve 12, oxamate 10 m*M* at 0 time, 11 m*M* excess NaCl at 30 min (11 atria).

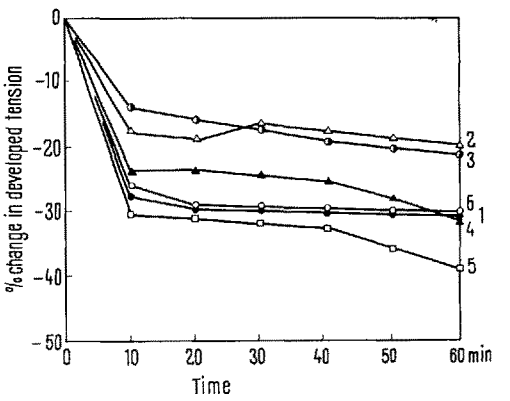

Fig. 2. Effects of oxamate and of oxamate plus lactate or pyruvate on normal atria. Curve 1, oxamate 10 m*M* at 0 time (9 atria); curve 2, oxamate 10 m*M* + 5.5 m*M* lactate at 0 time (5 atria); curve 3, oxamate 10 m*M* + 2.75 m*M* pyruvate at 0 time (5 atria); curve 4, oxamate 10 m*M* + 5.5 m*M* pyruvate at 0 time (5 atria); curve 5, oxamate 10 m*M* + 11 m*M* pyruvate at 0 time (5 atria); curve 6, oxamate 10 m*M* + 11 m*M* lactate at 0 time (6 atria).

[1] B. W. NOVOA, A. D. WINER, A. J. GLAID, and G. W. SCHWERT, J. biol. Chem. *234*, 1143 (1959).
[2] J. PAPACONSTANTINOU and S. P. COLOWICK, J. biol. Chem. *236*, 278 (1961).
[3] J. PAPACONSTANTINOU and S. P. COLOWICK, J. biol. Chem. *236*, 285 (1961).
[4] G. R. MCKINNEY and S. P. MARTIN, Blood *11*, 455 (1956).
[5] A. L. GIMENO, J. L. LACUARA, M. F. GIMENO, E. CERETTI, and J. L. WEBB, Molec. Pharmac. *2*, 77 (1966).
[6] A. L. GIMENO, M. F. GIMENO, E. A. SAVINO, and A. S. BEDNERS, Proc. Soc. exp. Biol. Med., in press (1966).
[7] W. C. YANG, Am. J. Physiol. *205*, 781 (1963).
[8] A. L. GIMENO, M. F. GIMENO, and J. L. WEBB, Am. J. Physiol. *203*, 1962 (1962).

to lactate? Inhibition of lactate dehydrogenase by oxamate results in increased cellular pyruvate levels, reduced glucose uptake and glycolysis (possibly related to a fall in NAD and ATP), and accumulation of fructose-1,6-diphosphate, as demonstrated in other tissues[9,10]. On the other hand, high concentrations of pyruvate have been shown to inhibit glycolysis in the perfused rat heart[11,12], and we[5,6] as well as others[7] have provided evidence that glucose uptake or utilization plays a definite role in the maintenance of atrial contractility, over and above the formation of substrate for the tricarboxylate cycle. Furthermore, high pyruvate concentrations depress atria despite the increase in ATP levels[5,6].

It is difficult to explain all the effects of oxamate, particularly the initial rapid fall in contractility, on the basis of a direct or indirect inhibition of glycolysis, resulting from changes in tissue pyruvate or citrate levels, or from an alteration of the cytoplasmic NAD:NADH ratio as a consequence of a supression of the conversion of pyruvate to lactate. It must be noted that the nature and time course of atrial depression with oxamate are different from those observed in glucose-free medium, during inhibition of glycolysis by 2-deoxyglucose, or in the presence of high pyruvate concentrations. On the other hand, if a fall in cytoplasmic NAD plays a role in the functional effects of the inhibitor, it would be reasonable to expect that the addition of lactate, the conversion of which to pyruvate is not likely to be affected by 10 m*M* oxamate[1,13], would result in further reduction of atrial contractility. Possibly the initial transient reduction of the rate of cellular depolarization is involved in the early phase of contractile failure by slowing the activation of contractile units. Later on some other factors might presumably come into the action maintaining atrial tension at a constant depressed level.

Pyruvate at 5.5–11 m*M* enhances the oxamate depression of atria in normal glucose medium; the depression is actually greater than in the absence of the inhibitor. This effect is not observed in glucose-free medium, either alone or with oxamate, and indeed under such conditions pyruvate induces a partial but definite recovery. On the other hand, pyruvate is unable to imporve contractility when added after 2-deoxyglucose and oxamate in the presence of glucose. It thus appears that the recovery with high pyruvate concentrations is lost when glucose is present, whether glycolysis is blocked by 2-deoxyglucose or not. Oxamate sensitizes the atria to the depressant effects of high pyruvate. These findings seem to be consistent with the idea that oxamate causes an intracellular accumulation of pyruvate in such a way that externally added pyruvate can more easily inhibit the initial steps of glycolysis, even at the glucose transport level, which may be involved in the regulation of atrial contractility.

It may be concluded that even though we have no definitive evidence to relate the principal effect of oxamate on the atria to an alteration of glycolysis, it seems clear that oxamate can sensitize the tissue to functional depression through glycolytic impairment. Inasmuch as it has been shown that oxamate competitively inhibits the reductive amination of pyruvate to alanine[14], an action which also favors the intracellular accumulation of pyruvate, it is possible that some of the effects of oxamate are related to alterations in atrial amino acid metabolism[15].

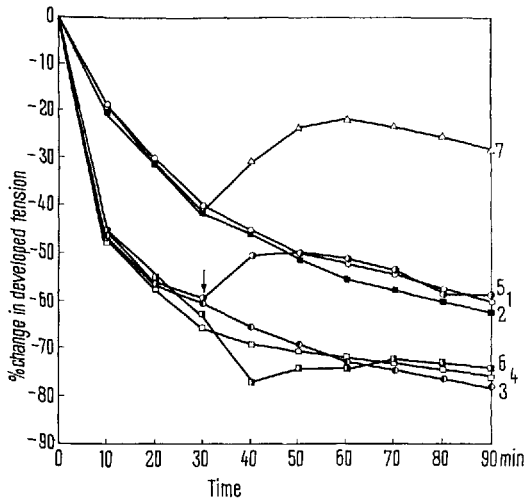

Fig. 3. Effects of oxamate on atrial responses to 2-deoxyglucose or absence of glucose. Curve 1, glucose-free medium at 0 time (7 atria); curve 2, 2-DG 10 m*M* in glucose medium at 0 time (7 atria); curve 3, glucose-free medium + 10 m*M* oxamate at 0 time (5 atria); curve 4, 2-DG 10 m*M* + 10 m*M* oxamate in glucose medium at 0 time (5 atria); curve 5, glucose-free medium + 10 m*M* oxamate at 0 time, 11 m*M* pyruvate at 30 min (5 atria); curve 6, 2-DG 10 m*M* + 10 m*M* oxamate in glucose medium at 0 time, 11 m*M* pyruvate at 30 min (5 atria); curve 7, glucose-free medium at 0 time, 11 m*M* pyruvate at 30 min (10 atria).

Zusammenfassung. Oxamat, das die Lactat-Dehydrogenase hemmt, erniedrigt die Kontraktionsfähigkeit des Vorhofs mit und ohne Anwesenheit von Glucose im Medium und bei Hinzufügung von 2-DG oder Atropin. Hohe Konzentration von Pyruvat erhöht die Depression von Oxamat auf den Vorhof im Glucose-Milieu. Oxamat sensibilisiert das Gewebe für funktionellen Abbau durch Glycolyse.

E. A. Savino[16], M. F. Gimeno[17], and A. L. Gimeno[17]

Segunda Cátedra de Fisiología, Universidad de Buenos Aires, Escuela de Medicina and Department of Pharmacology, School of Medicine, University of Southern California, Los Angeles (California 90033, USA), August 30, 1966.

[9] E. B. Goldberg and S. P. Colowick, J. biol. Chem. *240*, 2786 (1965).
[10] E. B. Goldberg and S. P. Colowick, J. biol. Chem. *240*, 2791 (1965).
[11] J. R. Williamson, J. biol. Chem. *240*, 2308 (1965).
[12] P. B. Garland, P. J. Randle, and E. A. Newsholme, Nature *200*, 169 (1963).
[13] B. W. Nova and G. W. Schwert, J. biol. Chem. *236*, 2150 (1961).
[14] E. Kun and B. Achmatowicz, J. biol. Chem. *240*, 2619 (1965).
[15] This research was supported by grants No. 1441 and No. HE-994801 from Consejo Nacional de Investigaciones de la República Argentina, and from the National Institute of Health, respectively.
[16] Research Fellow from the Consejo Nacional de Investigaciones de la República Argentina.
[17] Established Investigator from the Consejo Nacional de Investigaciones de la República Argentina. Present address: Department of Pharmacology, University of Southern California, School of Medicine.