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LIPOLYSIS AFTER NITROGLYCERINE AND ITS INHIBITION BY PROPRANOLOL

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Abstract—After giving 0·5 mg nitroglycerine sublingually to 20 healthy subjects there was an increase in plasma FFA from 603 ± 127 μmoles/l. to a maximum of 829 ± 232 μmoles/l. ($P < 0.001$). 120 min later the plasma FFA level was still 665 ± 160 μmoles/l., showing a significant increase over initial values ($P < 0.01$). Giving 2 mg propranolol intravenously to 15 healthy subjects prevented the increase in lipacidaemia after nitroglycerine. The intravenous injection of 2 mg propranolol alone had no effect on the plasma FFA level in 10 healthy subjects.

NITROGLYCERINE has been used in patients with angina pectoris since 1859 and its effect on the circulation is described in textbooks of pharmacology.[1] This effect of nitroglycerine is connected with a rapid change of the sympathetic nervous system tension, which leads to tachycardia and an increase in blood pressure. Since the catecholamines are the mediators of sympathetic stimulation we attempted to demonstrate their beta-stimulatory metabolic action, which is mediated by the adenylcyclase system, by the activation of hormone sensitive lipase and the subsequent liberation of fatty acids.[2,3] If it is true that lipolysis occurs after administration of nitroglycerine as a response to adrenergic stimulation, the administration of beta-blocking agents should prevent it.

The present investigation was an attempt to verify this assumption and therefore (a) a follow up of lipacidaemia was made after the administration of nitroglycerine and (b) a study was made of the effect of beta-blocking agents on changes in the free fatty acid level (FFA).

MATERIAL AND METHODS

Twenty subjects were investigated (13 men and 7 women), average age 51 years. None of them had a disturbance in fat or carbohydrate metabolism or cardiovascular disease. The investigation was made at rest in the morning after 12 hr fasting. A blood sample was withdrawn and 0·5 mg nitroglycerine (Nitroglycerine Spofa) given sublingually. Blood samples were then withdrawn 5, 10, 15, 30, 60 and 120 min after the first one. Blood samples were immediately elaborated.

An investigation was also made on 15 healthy subjects (7 men and 8 women), average age 47 years. Blood was collected from all these subjects and immediately afterwards 2 mg of the beta-blocking agent, propranolol (Inderal), were injected intravenously during 5 min. Ten minutes later 0·5 mg nitroglycerine was given perlingually so that the beta-blocking agent acted for 10 min before the application of the

nitroglycerine. Blood was then withdrawn at the same time intervals as in the first group.

Ten healthy subjects (5 men and 5 women), average age 46 years, acted as controls. They were given 2 mg Inderal intravenously only and blood samples were taken at the same time intervals.

FFA were estimated using Novak's photocolorimetric micromethod.[4] The Student *t*-test was used for statistical evaluation.[5]

RESULTS

In the first group the FFA level increased in all subjects after the application of nitroglycerine. The middle part of Fig. 1 shows the average FFA levels at the various time intervals: the smallest increase was 50 μmoles/l., the largest 640 μmoles/l., the average increase 226 μmoles/l. The height of the FFA increase after nitroglycerine had no relationship whatsoever to the initial FFA level.

The time of the maximum increase differed, occurring already 5 min after the administration of the drug in one subject, 10 min in 10 subjects, 15 min in 8 subjects and 30 min in one subject. At 120 min the FFA level had decreased to the initial value in one subject only.

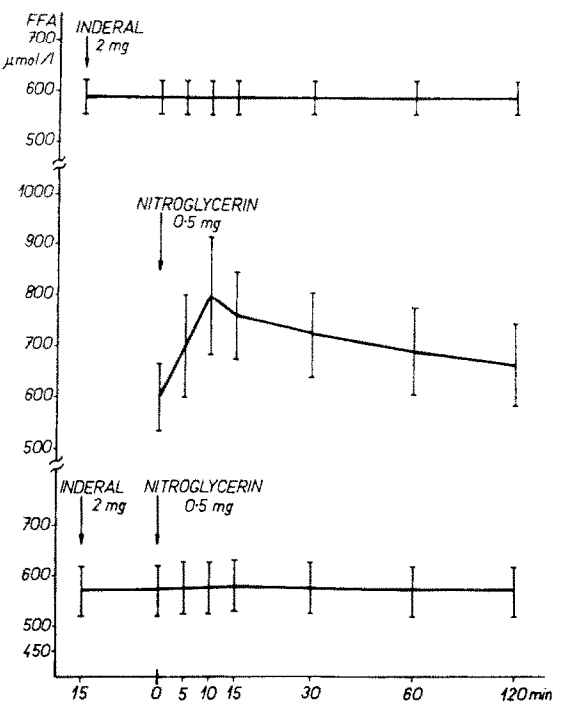

FIG. 1. The upper part of the graph shows average lipacidaemia values ($n = 10$) after giving 2 mg Inderal intravenously. The middle part of the graph shows average lipacidaemia values ($n = 20$) after giving 0·5 mg nitroglycerine at 0 min. The lower part of the graph shows average lipacidaemia values ($n = 15$) after giving 0·5 mg nitroglycerine at 0 min and 2 mg Inderal intravenously 15 min before giving nitroglycerine.
The standard deviations are shown with vertical lines.

The average initial FFA value was 603 ± 127 μmoles/l., the average maximum value (irrespective of time interval) was 829 ± 232 μmoles/l., a difference which is at the 0·1 per cent level of significance ($t = 6{\cdot}295 > 3{\cdot}883$). The average FFA level at 120 min was 665 ± 160 μmoles/l. and differed from the initial value at the 1 per cent level of significance ($t = 3{\cdot}375 > 2{\cdot}861$).

The lower part of Fig. 1 shows the average FFA values after nitroglycerine preceded by the administration of propranolol. In 5 subjects the FFA level did not change, in 10 it increased from an initial average of 571 ± 97 μmoles/l. to an average maximum value of 581 ± 101 μmoles/l. Thus, the average maximum increase was 10 μmoles/l. The average plasma FFA value at 120 min was 573 ± 97 μmoles/l. No statistical evaluation was made because the difference between the average initial and average maximum value did not exceed 2 per cent of the initial value, so that the changes found are within the range of technical error.

The upper part of Fig. 1 shows the average FFA values after the administration of propranolol. The initial average value was 586 ± 61 μmoles/l. In 3 subjects a decrease in lipacidaemia occurred, in 6 an increase, and 1 showed no change in the level during the investigation. The average FFA value at 120 min. was 586 ± 61 μmoles/l. The changes in lipacidaemia which did occur were within the limits of technical error (5–15 μmoles/l.) and therefore not statistically significant.

DISCUSSION

Recently, the question of the relationship between plasma FFA level and disturbances in the heart after myocardial infarction have come increasingly to the forefront. Kurien and Oliver observed considerably increased plasma FFA levels in patients with acute myocardial infarction and vascular cerebral episodes.[6] These authors further showed that in patients with myocardial infarction there was a higher incidence of arrhythmias, disturbances in conduction and even deaths if the FFA level rose to above 1000 μmoles/l.[7] They consider that the relationship between increased FFA level and arrhythmias is either the result of increased catecholamine activity, mainly that of noradrenalin, or is the direct result of increased oxygen consumption by the myocardium due to the utilization of FFA. In either case, however, they conclude that there would be increased myocardial hypoxia.[8] They combine their ideas in the hypothesis that the combined effect of myocardial hypoxia, the catecholamines liberated during myocardial infarction[9] and increased FFA level, lead to arrhythmias, the main part being played by the detergent action of FFA on cell membranes which leads to disturbances in ionic balance, this being the immediate cause of disturbances in heart rhythm.[8]

They managed to demonstrate the truth of their hypothesis about the unfavourable effect of increased plasma FFA levels on the myocardium in animal experiments. They activated lipoprotein lipase by heparin in dogs with acute myocardial infarction and found that the subsequent increased FFA levels led to the occurrence of ventricular arrhythmias.[10] As shown by Hoak *et al.*,[11] an increased FFA concentration can damage myocardial cells or according to Challoner *et al.*[12] lead to increased oxygen consumption.

Henderson *et al.*, on the other hand, point out that it has never been definitely demonstrated that there is a relationship between increased plasma FFA levels and

increased oxygen consumption in the myocardium. They found, on the contrary, that high plasma FFA levels decreased myocardial oxygen consumption and increased coronary vascular resistance in the isolated rat heart under constant perfusion.[13] Nor have clinical observations of other investigators confirmed the conclusions of Oliver and Kurien concerning the relationship between increased plasma FFA levels and a higher incidence of arrhythmias in patients with acute myocardial infarction.[14–19]

Although the opinion of Kurien and Oliver is not universally accepted, it is necessary to bear in mind the danger which could be caused by a drug which increases lipolysis or which liberates catecholamines with their known lipomobilizing effect. We were, therefore, interested in whether the administration of nitroglycerine alters the plasma FFA level. We found only one reference in the literature and that was the observation of a slightly increased FFA level in dogs after the injection of nitroglycerine.[20] We recorded a marked increase in plasma FFA levels in healthy subjects after nitroglycerine given perlingually. When a beta-blocking agent was given before the nitroglycerine the FFA levels did not deviate from initial values during the course of the investigation. These results confirm our assumption that nitroglycerine liberates catecholamines with their lipolytic effect. After giving a beta-blocking agent their beta-stimulating action is paralysed, lipolysis does not increase and the plasma FFA level remains unaltered.

The inhibition of the increase in FFA by propranolol or other beta-blocking agents after the stimulation of lipolysis by catecholamines has already been demonstrated and the reports in the literature on this fact are concordant.[21–23] Various authors obtained diverse results when determining the plasma FFA levels after the administration of the beta-blocking agent, propranolol, alone without previously stimulating lipolysis.

Thus Garattini and Bizzi[23] found no change in the FFA level in rats after propranolol, whereas Fassina observed an increase in FFA level also in rats.[24] This author further found that the increase is more marked after giving smaller doses (2 mg/kg subcutaneously), is less after higher doses (5 mg/kg s.c.) and the least after the highest doses of propranolol (40 mg/kg s.c.). Zsoter *et al.* did not find changes in the FFA level after propranolol in dogs,[25] whereas Nakano *et al.* found a slight decrease.[26] Kaufmann *et al.*[27] found a slight fall in FFA level in people taking propranolol in a dose of 40 mg/os. Other authors obtained similar results in man.[22] Our investigations showed that propranolol in a dose of 2 mg intravenously did not affect the plasma FFA level in the subjects investigated.

The diverse findings in relation to plasma FFA values after beta-blocking agents is explained on the basis of the assumed lipolytic effect on the one hand, and the antiadrenergic action of beta-blocking agents on adipose tissue, on the other. The mutual relationship of these two actions varies in dependence on the dose of beta-blocking agent and the initial plasma FFA level. At a low level the lipomobilizing effect is more marked, at a high FFA level (e.g. after stimulation of lipolysis by catecholamines) the antiadrenergic action comes into play.[24]

Our findings, however, are slightly different and do not fit with the above explanation. We consider that the dose of propranolol we used was too small to produce lipomobilization in adipose tissue, but that it was sufficient to block lipomobilization caused by beta stimulation, thereby preventing lipomobilization due to catecholamines that were liberated by nitroglycerine. The intravenous administration of 2 mg propranolol was without effect on lipacidaemia.

It can be said in conclusion that our results confirm that nitroglycerine increases the FFA level due to the liberation of catecholamines with their lipolytic action. After giving a beta-blocking agent this beta-stimulating metabolic effect of catecholamines does not occur, lipolysis is not increased and the plasma FFA level is unaltered. Propranolol alone did not affect the plasma FFA level.

REFERENCES

1. L. S. Goodman and A. Gilman, *The Pharmacological Basis of Therapeutics*, pp. 730–743. MacMillan, New York (1955).
2. R. W. Butcher and E. W. Sutherland, *Protein and Polypeptide Hormones, Excerpta Med. Intern. Congress* **161,** 150 (1968).
3. Y. Combrel, *Ph. Landat, Rev. franc. Étud. clin. Biol.* **13**, 329 (1968).
4. M. Novák, *J. lipid Res.* **6,** 431 (1965).
5. J. Janko, *Statistické tabulky*, Academia ČSAV, Praha (1958).
6. V. A. Kurien and M. F. Oliver, *Lancet* **II,** 122 (1966).
7. M. F. Oliver, V. A. Kurien and T. W. Greenwood, *Lancet* **I,** 710 (1968).
8. V. A. Kurien and M. F. Oliver, *Lancet* **I,** 813 (1970).
9. R. F. Klein, W. G. Troyer, H. K. Thompson, M. D. Bogdonoff and A. G. Wallace, *Arch. intern. Med.* **86,** 476 (1968).
10. V. A. Kurien, P. A. Yates and M. F. Oliver, *Lancet* **II,** 185 (1969).
11. J. C. Hoak, W. E. Connor and E. D. Warner, *J. clin. Invest.* **47,** 2701 (1968).
12. D. R. Challoner and D. Steinberg, *Am. J. Physiol.* **210,** 280 (1966).
13. A. H. Henderson and E. H. Sonnenblick, *Lancet* **I,** 1179 (1970).
14. H. L. Rutenberg, J. C. Pamintuan and L. A. Soloff, *Lancet* **II,** 559 (1969).
15. H. L. Rutenberg and L. A. Soloff, *Lancet* **I,** 198 (1970).
16. P. G. Nelson, *Br. Med. J.* **3,** 735 (1970).
17. P. G. Nelson, *Lancet* **I,** 783 (1970).
18. D. K. Gupta, D. E. Jewitt, R. Young, M. Hartog and L. H. Opie, *Lancet* **II,** 1209 (1969).
19. J. V. Russo, S. Margolis, G. C. Friesinger and R. S. Ross, *Lancet* **II,** 1271 (1970).
20. D. Steinberg and R. Pittman, *Proc. Soc. exp. Biol. Med.* **123,** 192 (1966).
21. D. C. Harrison and J. R. Griffin, *Circulation* **34,** 218 (1966).
22. E. J. Pinter and C. J. Pattee, *J. clin. Endocr. Metab.* **27,** 1441 (1967).
23. S. Garattini and A. Bizzi, *Pharmac. Rev.* **18,** 243 (1966).
24. G. Fassina, *J. Pharm. Pharmac.* **18,** 399 (1966).
25. T. Zsoter, H. Tom, M. Kraml and D. Dvornik, *J. Pharmac. exp. Ther.* **152,** 425 (1966).
26. J. Nakano, T. Kusakari and J. Berry, *Archs. int. Pharmacodyn.* **164,** 120 1966.
27. N. A. Kaufmann and S. Stern, *Israel J. Med. Sci.* **5,** 1002 (1969).