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Pharmacological properties and mechanism of action of atractyloside

SIR,—During the past eight years, we have investigated the biochemical and pharmacological properties of a natural glycoside, atractyloside, extracted from the rhizome of a Mediterranean member of the Compositae, *Atractylis gummifera* L. and much information has been obtained.

The toxic and therapeutic effects of this natural drug were well known in classical times, and are referred to in Dioscorides' *De Materia Medica* under the name of "Chamaeleon". Caccialanza & Landi (1960–1961) have confirmed that the use of this drug in skin diseases, as practiced in antiquity, was fully justified.

The pharmacological action of the drug can be attributed to a glycoside formed by an aglycone which has the structure of a perhydrophenanthrene, one molecule of glucose, two of potassium sulphate, and one of isovaleric acid (Ajello, Piozzi, Quilico & Sprio, 1963). The relation between chemical structure and pharmacological action of the drug is of interest (Santi, Bruni, Contessa & Luciani, 1962). We now summarise the results of our *in vivo* and *in vitro* investigations.

In rats, dogs, mice and rabbits atractyloside induces hypoglycaemia, usually preceded by a phase of hyperglycaemia, depletion of muscular and hepatic glycogen reserves (myocardial glycogen remains unchanged or is even slightly increased), and inhibition of glycogen synthesis. An increase in the blood lactic acid level and a decrease in oxygen consumption have also been observed (see Santi, 1958).

The effect elicited *in vivo* can be explained by the finding that the drug is a powerful inhibitor of oxidative phosphorylation in isolated mitochondria and in rat liver homogenate (see Santi, 1958). On this basis it may be presumed that the hypoglycaemic effect is connected with an inhibition *in vivo* of the Pasteur effect. The inhibition of glycogen synthesis as well as the inhibition of oxygen uptake and the increase in blood lactic acid are the consequence of the marked reduction in cellular oxidative and phosphorylation reactions. Subsequently, Bruni, Contessa & Luciani (1962) and Bruni & Luciani (1962) have shown that the atractyloside-induced inhibition of mitochondrial oxygen uptake is reversed by the uncoupling effect of 2,4-dinitrophenol, and in general by conditions in which the respiratory processes are not controlled by phosphorylation. Among the partial reactions of the coupling system, 2-4-dinitrophenol-stimulated adenosine-triphosphatase (ATPase), ATP-inorganic phosphate, and ATP-ADP exchanges are markedly inhibited by atractyloside, whereas Mg^{2+}-stimulated ATPase is influenced only if the activity is elicited by a partial damage of mitochondria; no effect whatever is induced if mitochondrial structure is severely disorganised.

These results have led us to conclude that atractyloside inhibits oxidative phosphorylation by a process of specific interference with energy transfer reactions.

Recently, the analysis of the mechanism of action of atractyloside on mitochondrial energy transfer has been brought a step forward by the finding

that a competitive inhibition can be shown to exist between atractyloside and ADP. These studies have led to the conclusion that atractyloside acts on the terminal phase of energy transfer, the phase in which a high-energy phosphorylated intermediate, originating from inorganic phosphate and the energy-conserving reaction of the respiratory chain, forms ATP from ADP. This assumption is supported by the following observations (Bruni, Luciani & Contessa, 1964; Bruni, Luciani, Contessa & Azzone, 1964): first, atractyloside specifically inhibits the binding of ADP and ATP to liver mitochondria; second, in contrast to the inhibition induced in respiration stimulated by inorganic phosphate (P_i) and P_i-acceptor, atractyloside does not affect arsenate-stimulated respiration, which must be ascribed to the splitting of the unstable arsenilated compound formed instead of a stable phosphorylated analogue; thus, atractyloside does not interfere with the entry of As (or of P_i) into energy transfer reactions. Third, atractyloside inhibits the substrate level phosphorylation linked to the oxidation of α-ketoglutarate. It is interesting to note that the energy transfer inhibitor oligomicin (Lardy, Johnson & McMurray, 1958), which acts on the interaction of P_i with the coupling system, inhibits arsenate-induced respiration but does not affect substrate level phosphorylation.

The lack of sensitivity to atractyloside of Mg^{2+}-stimulated ATPase in severely damaged mitochondria does not invalidate this hypothesis, since these mitochondrial preparations have lost the capacity to bind atractyloside. Analysis of atractyloside effect on oxidative phosphorylation, ATPase stimulated by 2,4-dinitrophenol or by low concentrations of sodium deoxycholate shows a competitive removal of inhibition by increasing the amounts of adenine nucleotides in the reactions involving both ADP and ATP. From these results it was concluded that the mechanism of the inhibitory effect on the terminal step of the energy transfer process, may be explained by admitting a competitive inhibition between adenine nucleotides and atractyloside on the same mitochondrial receptor site.

It seems reasonable to expect that atractyloside will acquire more importance as a biochemical tool because the most prominent feature of its action is selective inhibition of phosphorylation.

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