Azide Reversibly Raises Cyclic GMP Levels in Hepatic Slices and Activates Guanylate Cyclase*

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In vitro activation of hepatic quanylate cyclase (E.C. 4.6.1.2) by sodium azide and by nitroso compounds such as *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and *N*-methyl-*N*-nitrosourea have been described five years ago [1,2] and the finding was expanded to several other tissues including kidney [3] and heart.[4] It was established that a protein factor (catalase),[5,6] that converts the nitroso compounds into NO, is required for the activation *in vitro* of guanylate cyclases. Our studies were based on the widely held assumption that the carcinogeneity of the nitroso compounds is dependent on modification of guanosine residues in DNA [7] and on the simultaneous effect of prolonged promotion of cell division *via* elevation of cGMP levels.[8] Our findings, however, indicate that azide elevates cyclic GMP levels only transiently.

Experimental. Liver slices (0.4 mm thickness) were prepared from adult male Spraque Dawley rats and incubated in normal Krebs-Ringer medium with or without the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX) (1 mM) for 15 min at 37 °C in a shaking waterbath. After 15 min incubation with IBMX, sodium azide (100 mM) was added to a final concentration of 1 mM.

The incubations were interrupted at different time intervals, the medium removed and 1 ml ice-cold perchloric acid (0.3 M) was added to the slices. Cyclic GMP was extracted according to Folbergova [9] and measured with a radioimmuno assay according to Steiner *et al.*[10]

Guanylate cyclase was assayed with MnGTP (1 mM) as substrate in triethanolamine.HCl buffer (50 mM), pH 7.6, as described previously.[11]

Results and discussion. The phosphodiesterase inhibitor, IBMX raises cyclic GMP levels in hepatic slices. This level is constant during the 60 min incubation. Addition of NaN_3 (1 mM) raises cyclic GMP levels both in the absence and in the presence of IBMX. The cGMP levels elevated by NaN_3 persisted only for 15–30 min and declined later towards the basal cyclic GMP level (Fig. 1).

Using 1 mM MnGTP as substrate concentration, the time course of the activation by NaN_3 (1 mM) was examined in liver homogenates. Fig. 2 shows that activation of guanylate cyclase by NaN_3 persists for at least 90 min.

The results presented here confirm that cyclic GMP levels and guanylate cyclase activity are raised by incubation with NaN_3 (1 mM). The main finding

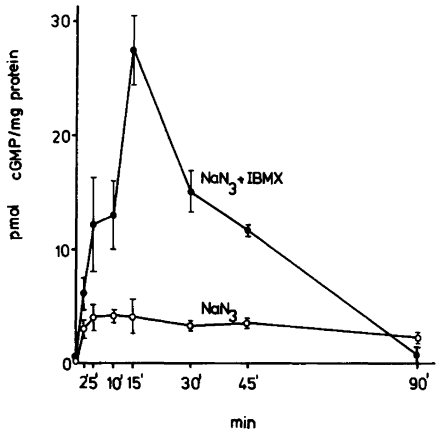

Fig. 1. The effects of 3-isobutyl-1-methylxanthine (1 mM) and of NaN_3 (1 mM) on cyclic GMP levels in liver slices. For experimental details, see Experimental. ○, NaN_3; ●, IBMX + NaN_3. Control levels were lower than 0.1 pmol/mg protein.

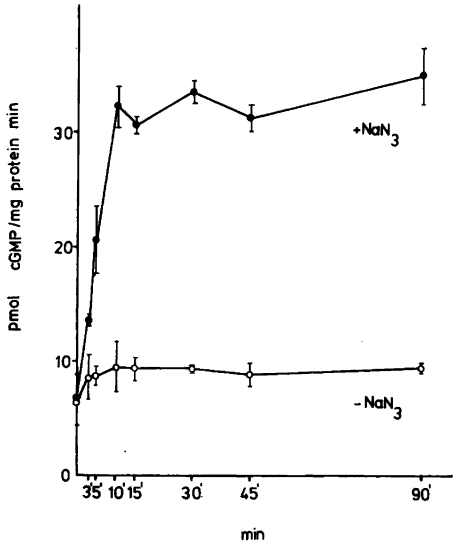

Fig. 2. The time course of activation of guanylate cyclases in liver homogenate by NaN_3 (1 mM) at Mn-GTP (1 mM), free Mn^{2+} (3 mM). ○, without NaN_3; ●, with NaN_3.

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of this study is that intact cells, such as those in liver slices, possess mechanisms to reduce cGMP levels after or despite of the activation by NaN_3. Similar results were found in hepatocytes [12] where lowering of elevated cGMP levels is achieved by pumping out cGMP. Thus, elevation of cyclic GMP levels is short-lived unlike the *in vitro* activation of guanylate cyclase [1-3] (*cf.* Fig. 2). It is possible that raising cyclic GMP levels even for a short period of 15–30 min is sufficient to produce long-lasting effects on cell division and other processes. There are examples of short pulses of hormones having profound long-lasting effects.[13]

The findings presented here indicated that we have to concentrate on a relatively short period after exposure to NaN_3 if we want to study and prevent the effects of these compounds on cyclic GMP levels and on the subsequent events triggered by cyclic GMP.

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