Changes in the concentration of vasoactive intestinal peptide in intestinal lymph in response to vagal stimulation in the calf[1]

A. V. Edwards, P. M. M. Bircham, S. J. Mitchell and S. R. Bloom

Physiological Laboratory, Downing Street, Cambridge CB2 3EG (England) and Department of Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, London W12 (England), 8 February 1978

Summary. Stimulation of both vagi caused a significant rise in arterial plasma vasoactive intestinal peptide (VIP) concentration in 3–5-week-old calves with cut splanchnic nerves. This was associated with a pronounced rise in the VIP concentration of intestinal lymph showing that vagal stimulation causes release of VIP from splanchnic viscera.

Stimulation of the peripheral ends of the thoracic vagi causes a rise in the concentration of vasoactive intestinal peptide (VIP) in the circulating plasma of the pig[2]. However, it is not yet known what physiological effects, if any, are mediated by this peptide, nor whether it is capable of acting hormonally. The finding that VIP is present in nerve terminals in the gut[3–5] is suggestive of a more localised role, in which case changes in the concentration of the peptide in the circulating plasma may merely reflect the rate at which it is washed out of the tissue. Accordingly, we have investigated the changes in VIP concentration in intestinal lymph, in response to vagal stimulation, in order to obtain more direct evidence about the concentration of the free peptide in the tissue.

Methods. The experiments were carried out in 12 pedigree Jersey calves (24–41 kg b.wt), 3–5 weeks after birth, which were anaesthetized by i.v. injection of sodium pentobarbitone (Sagatal, May and Baker; 20 mg/kg). Food was witheld for at least 14 h prior to each experiment. The peripheral ends of the thoracic vagi were attached separately to fluid electrodes and stimulated at 10.0 Hz using supramaximal stimuli (20 V; 1 msec square-wave pulses) for 10 min. When required both splanchnic nerves were cut just below the diaphragm. A cannula was inserted into the intestinal duct, as it entered the cisterna chyli for subsequent collection of intestinal lymph. Samples of lymph and arterial plasma for VIP estimations were treated with aprotonin and stored at −20 °C. VIP was measured by radioimmunoassay[6].

Results and discussion. Stimulation of the peripheral ends of both vagus nerves immediately above the diaphragm at 10.0 Hz for 10 min caused a significant rise in the concentration of VIP in the arterial plasma in 3–5-week-old calves with cut splanchnic nerves (figure 1). Mean arterial plasma VIP concentration rose steadily after a delay of 2.5 min to a peak incremental value of 22±7 pmoles/l at 12.5 min and subsided slowly thereafter. There was also a significant fall in mean aortic blood pressure from 106±4 mm Hg initially to 85±6 mm Hg at 2.5 min ($p < 0.02$) and 86±4 mm Hg at 10 min ($p < 0.01$) together with a rise in mean heart rate. In calves with intact splanchnic nerves there was much wider variation between the arterial plasma VIP concentration of different individuals and no consistent change occurred in response to vagal stimulation (figure 1). Mean aortic blood pressure had fallen from 105±5 to 77±9 mm Hg after 2.5 min. It therefore seems likely that reflex increase in sympathetic activity, consequent upon vagally induced hypotension, interferes in some way with the VIP response to vagal stimulation.

In calves with cut splanchnic nerves vagal stimulation produced a signficant rise in the flow of intestinal lymph together with the concentration of VIP therein (figure 2). Computation of the output of VIP from the splanchnic viscera via the lymphatic system in response to vagal stimulation shows a) that there is a highly significant increase in statistical terms (figure 2; $p < 0.001$) and b) that the total amount of VIP entering the circulation via the lymphatic system is trivial in comparison to that which must enter directly through the capillaries in the splanchnic bed.

The rise in the concentration of VIP in the intestinal lymph, which we assume reflects the concentration of the peptide in the alimentary tract, must be attenuated by 2 factors. First the dead space of the intestinal lymphatic system which will delay the response. Secondly, the fact that the intestinal duct drains the liver, pancreas and spleen in addition to the alimentary tract. These viscera were virtually devoid of VIP in the calf (S.R. Bloom, A.V. Edward, S.J. Mitchell and L.B. Miller, unpublished observations). Their total weight was 28.3±10.9 g/kg while that of the alimentary tract was 56.4±2.3. In occasional animals, in which it has proved possible to cannulate the hepatic and intestinal

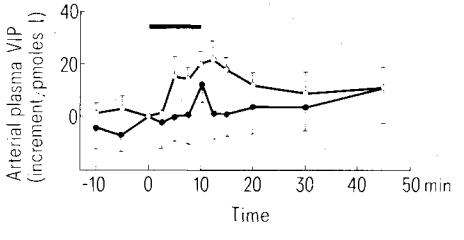

Fig. 1. Comparison of the changes in mean arterial plasma VIP concentration of 2 groups of calves 3–5 weeks after birth in response to stimulation of the peripheral ends of both vagus nerves at 10.0 Hz for 10 min. O—O cut splanchnic nerves, n = 6. ●—● intact splanchnic nerves, n = 6. Vertical bars: SE of each mean value. Horizontal bar: duration of stimulation. Mean basal values as follows. Calves with cut splanchnic nerves 17±7 pmoles/l. Calves with intact splanchnic nerves: 17±5 pmoles/l.

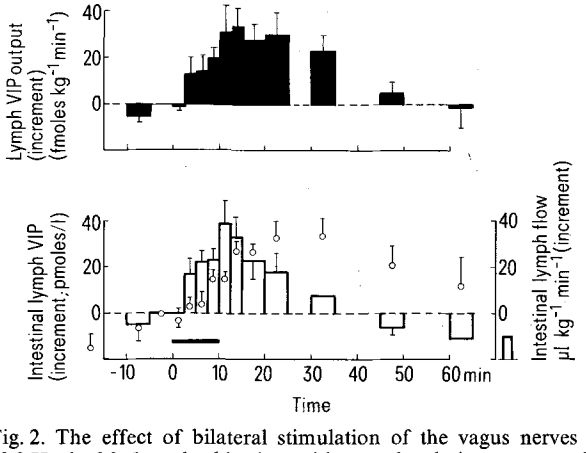

Fig. 2. The effect of bilateral stimulation of the vagus nerves at 10.0 Hz, in 6 3–5-week-old calves with cut splanchnic nerves, on the flow of intestinal lymph and the concentration of lymphatic VIP (below) together with the output of VIP in the intestinal lymph (above). Vertical bars: SE of each mean value. Horizontal bar: duration of stimulus. Mean basal values as follows: lymph flow: 44±6 $l \cdot kg^{-1}$ min^{-1}. VIP concentration: 34±13 pmoles/l. VIP output 18±9 pmoles kg^{-1} min^{-1}.

tributaries separately, we have found the lymph formation occurs at about the same rate, when it is expressed as flow per unit weight, in all these different tissues. A better estimate of the mean rise in the concentration of VIP in the intestinal extracellular fluid in response to vagal stimulation may therefore be obtained by correcting for estimated dilution by hepatic, splenic and pancreatic lymph. The peak incremental value (34±7 pmoles/l at 30 min) would then become 34.0 (84.7/65.4)= 51 pmoles/l, which is much greater than the observed rise in the concentration of the peptide in the arterial plasma. We conclude that the rise in VIP concentration in the blood which occurred in these experiments, in response to vagal stimulation, was due to release of the peptide from the alimentary tract.

1 Acknowledgment. This work has been supported by grants from the Agricultural Research Council and the Medical Research Council and we are indebted to Dr D.M. Burley (CIBA) for his continued support.
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Response of neonatal mouse lung in organ culture to silica

H. Yoshihara and D. Yew

Pasadena College of Chiropractic, Pasadena (California 91101, USA) and Department of Anatomy, University of Hong Kong, 5 Sassoon Road, Hong Kong (Hong Kong), 15 February 1978

Summary. Short term organ cultures of lung fragments from neonatal mice maintain their normal architecture. Cultures exposed to silica flour in varying concentrations show effects resembling those seen in chronic silicosis produced by inoculation and inhalation methods.

Although the effects of silica on lungs in vivo and in cultures of macrophages have been reported[1-3], the effects of silica on organ cultures of lung have not been documented. Animal experiments using intrapleural inoculation and inhalation methods are time consuming. The purpose of this study was to determine if an organ culture method could be used to reproduce the in vivo picture of chronic silicosis in a short period of time.

Materials and methods. Fragments of lung from neonatal (2-3 day) mice of strain NMR/BOM were cultured by the watch glass method[4] in medium TC 199[5] supplemented with 25% mouse plasma. Sterile silica flour containing 99.8% SiO_2 with a pH of 6.9 was dissolved in concentrations of 10%, 50% and 75% in distilled water. Control cultures received 15 drops of culture medium plus 3 drops of distilled water. Experimental explants received 15 drops of culture medium plus 3 drops of silica solution. Incubation took place in a Thelco incubator supplemented with 21% O_2, 8% CO_2, and 71% N_2. The culture medium was changed once during the 4-day incubation period.

Results. Control explants showed normal lung architecture comparable to lungs of mice of 2-3 days of age. Explants incubated with 10% silica flour for 4 days displayed pyknotic cells, migration of fibroblasts and macrophages and a decrease in size of alveolar spaces. Thin fibres and patches of eosinophilic material were evident in the alveolar spaces. The effects were progressively more severe with higher doses of silica. In fragments incubated with 75% silica flour the alveolar spaces were insignificant. Thin fibres were also present. The eosinophilic material was masked by the intense cellular infiltration.

Discussion. The results obtained in this study are similar to those reported in animal experiments[1-6]. The amorphous eosinophilic material found in the alveolar spaces have been reported in silica induced alveolar lipoproteinosis[7]. The migrating cells should be macrophages, the key cellular element in the fibrosis reaction[8-9]. Perhaps the macrophages are converted to fibroblasts as observed in aging lung cultures from adult guinea-pigs[10]. Short term organ cultures utilizing asbestos have been reported[11,12]. The experiments reported here with silica flour show that the in vivo picture of silicosis may be reasonably reproduced in a short incubation period.

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