Changes in GH and PRL release, produced as a result of APM administration, were not due to the emetic effect of the drug, because no emesis occurred in animals after drug treatment. Further work is needed to define the precise mechanism(s) whereby APM acts on GH and PRL release in cattle.

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PRO EXPERIMENTIS

Simultaneous measurement of pyridine nucleotide fluorescence and field potentials from the olfactory cortical slice of the guinea-pig

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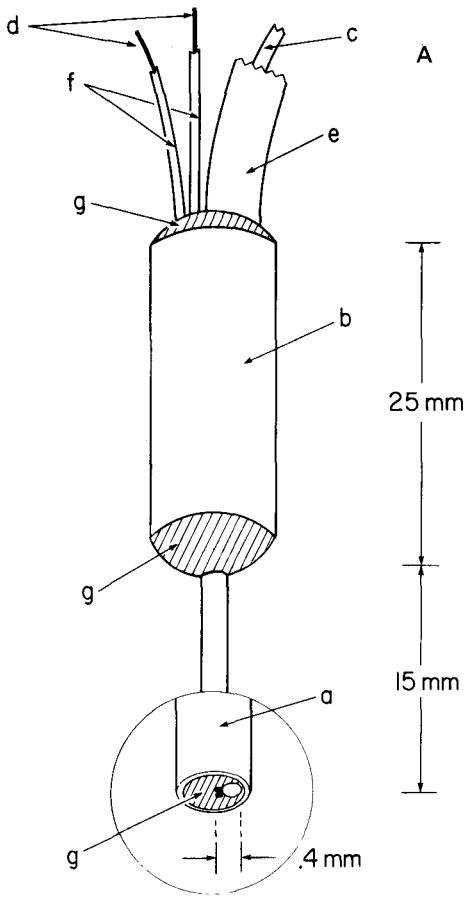
Summary. A new probe (tip diameter = 700 µm) consisting of a micro-light guide and a silver wire electrode was constructed to measure pyridine nucleotide fluorescence and field potentials from nerve tissues. Good kinetic correlations were found between the changes in field potentials and tissue pyridine nucleotide fluorescence caused by stoppage or by resumption of superfusion.

Since Chance and his co-workers introduced the tissue pyridine nucleotide (NADH) fluorometric technique in the late 1950's[2], several investigators have applied the method to the study of biochemical events occurring in nerve tissues under electrically-induced depolarizing conditions[3-7]. The present study was undertaken to measure simultaneously the electrical activity and the NADH fluorescence changes from the olfactory cortical slice of the guinea-pig caused by stoppage or resumption of superfusion. For this purpose we have developed a new probe consisting of a microlight guide[8,9] and a Ag-AgCl electrode (figure 1, A). Using this combination probe, we were able to measure the field potential and NADH fluorescence signals simultaneously and from the same tissue areas approximately 400 µm in diameter (figure 1, A).

Materials and methods. The method for producing the combination probe is basically the same as that for constructing a micro-light guide[8,9]. Figure 1, A shows schematically the structure of the combination probe. The Ag-AgCl wire was carefully insulated from the steel tubing. The optical fibres were connected to a 'DC' fluorometer[10] of the Johnson Research Foundation, University of Pennsylvania, and the silver wire to an oscilloscope.

The brain slices (0.4-0.5 mm thick) were prepared from the olfactory cortex of guinea-pigs, and the field potentials were measured under the 'gas-blow and medium-flow' conditions developed by Fujii et al.[11,12]. In order to stabilize the optical signal, it was necessary to place a strip of black polyethylene membrane (0.13 mm × 1.2 mm × 18 mm) beneath the brain slice as shown in figure 1, B, and to position the tip of the combination probe on that portion of the brain slice whose opposite surface was in contact with the black membrane. This arrangement eliminated the fluctuation of the NADH fluorescence signal which was apparently caused by periodic variations of the thickness of the Krebs-Ringer solution layer underneath the brain slice. Supramaximal stimulation was applied to the anterior part of the lateral olfactory tract (LOT). The temperature of the brain slice was continuously measured with a thermistor (0.7 mm in diameter, insulated with thin glass). The composition of the incubation medium (Krebs-Ringer's solution) was (mM): NaCl (120); KCl (4.8); KH_2PO_4 (1.2); $MgSO_4$ (1.2); $CaCl_2$ (2.6); $NaHCO_3$ (26); glucose (10); pH adjusted to 7.4 with bicarbonate buffer. The medium was equilibrated with a 5% CO_2 and 95% O_2 gas mixture.

Results. It was found that the NADH fluorescence intensity of the brain slice was very sensitive to temperature alterations. Figure 2 shows a typical temperature sensitivity curve of the olfactory cortical slice. From 4 brain slices, the average tissue NADH fluorescence intensity was found to be 101.9 ± 0.4% (mean ± SD) at 35 °C (relative to the fluorescence intensity at 37 °C set at 100%), 107 ± 1.3% at 30 °C, 114.2 ± 2.0% at 25 °C, and 121.2 ± 1.5% at 20 °C. The high temperature sensitivity of the NADH fluorescence intensity is not unique to the brain slice, since similar temperature sensitivity was also observed with isolated, hemoglobin-free perfused rat liver and an aqueous solution of NADH (S. Ji and T. Fujii, unpublished observations).



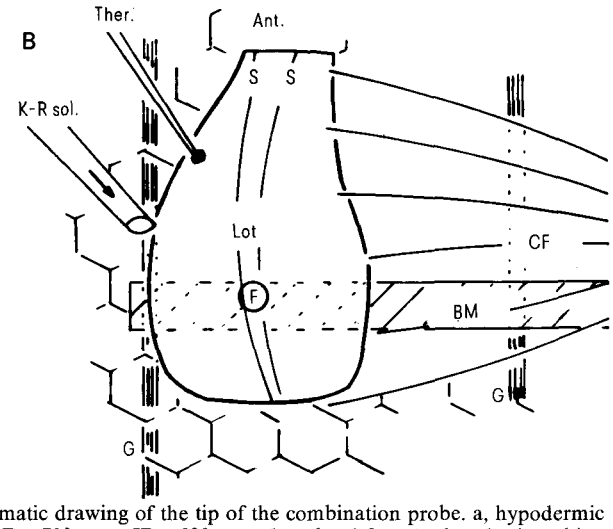


Fig. 1. *A* A schematic drawing of the tip of the combination probe. a, hypodermic steel tubing (OD = 700 µm, ID = 500 µm, length = 1.5 cm); b, plastic tubing (OD = 2.2 mm, ID = 1.6 mm, length = 2.5 cm); c, silver wire (diameter = 300 µm, length = 18 cm); d, glass-clad, glass-core optical fibre (American Optical Co., Southbridge, Mass.; diameter = 80 µm, length = 55 cm, acceptance half-angle = 15.1° in water); e, plastic tubing (OD = 1.2 mm, ID = 0.7 mm, length = 50 cm); f, hypodermic steel tubing (OD = 200 µm, ID = 140 µm, length = 50 cm); g, 5-min epoxy glue (Devcon Corporation, Danvers, Mass. 01923). The 2 dark circles are the optical fibres and the white circle is the silver wire. *B* A schematic drawing of the olfactory cortical slice. Krebs-Ringer's solution (K-R sol.) fell from a polyethylene tubing dropwise onto the upper surface of the brain slice and thereby superfused the whole slice before the medium either dropped into the reservoir under the nylon mesh or was sucked away through the cotton filaments (CF). The anterior (Ant.) part of the LOT was bipolarly stimulated (SS). The tip of the combination probe was placed on the brain slice at the position indicated by F. This area of LOT was wider than that of the tip of the probe. The temperature of the slice was continuously monitored with a thermistor (Ther.). BM, black membrane; G, grid of silver wire.

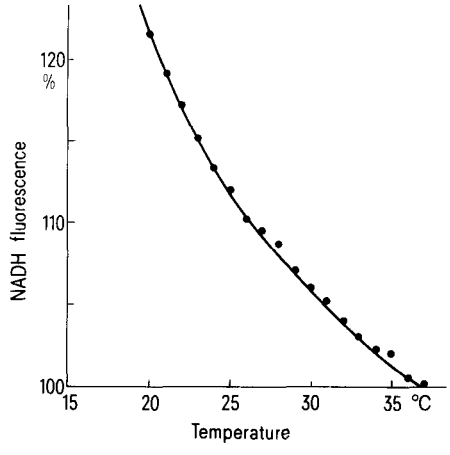


Fig. 2. The temperature dependency of the NADH fluorescence intensity of the olfactory cortical slice of the guinea-pig. The data points were calculated from the NADH fluorescence and temperature traces similar to figures 3, A and B, obtained during a continual change in the temperature of the superfusion medium at the rate of 1 to 0.5 °C/min. The other experimental details were similar to those described in the legend to figures 1, B, and 3, A.

When superfusion was stopped and the blowing gas was switched from 95% O_2 and 5% CO_2 to 95% N_2 and 5% CO_2, NADH fluorescence increased in a biphasic manner (figure 3, A). Upon resuming superfusion, NADH fluorescence decreased, again in a biphasic fashion. The small oscillations of the fluorescence signal were caused by the temperature variations of the brain slice (figure 3, B). Stopping superfusion caused the temperature of the brain slice to decrease toward the ambient temperature and the resumption of superfusion caused the temperature to rise. When we corrected the original fluorescence trace (figure 3, A) for the temperature sensitivity of NADH fluorescence using figure 2 as a calibration curve, the result shown in figure 3, C was obtained. This 'temperature-corrected' fluorescence change (ΔF_{tc}) reflects true redox biochemical changes occurring in the brain slice induced by stopping or resuming the oxygen supply to the tissue. The electrical activities of the brain slice were recorded by photographing the oscilloscope traces throughout the experiment. Some representative traces are displayed in figure 3, C. The half time of the decay of IS and N potentials was approximately 1 min and these potentials disappeared at 2.5 min after stopping superfusion. At this time, the temperature-corrected NADH fluorescence increased by approximately 3% of the basal fluorescence intensity. Thereafter fluorescence continued to increase, reaching a plateau value of 5.8% at about 6 min. The IS and N potentials began to reappear after approximately 1 min of resuming superfusion (4 in figure 3, C). The reappearance of the potentials was accompanied by a small decrease (1%) in the temperature-corrected NADH fluorescence. At 14 min after the resumption of superfusion, the temperature-corrected NADH fluorescence returned to the control level and the field potentials recovered partially, probably because of the rather long (6 min) anoxic period employed. Better recovery was observed with shorter anoxic cycles. The slow drift of the baseline in figure 3, A observed throughout the experiment was taken into account when the values in figure 3, C were calculated.

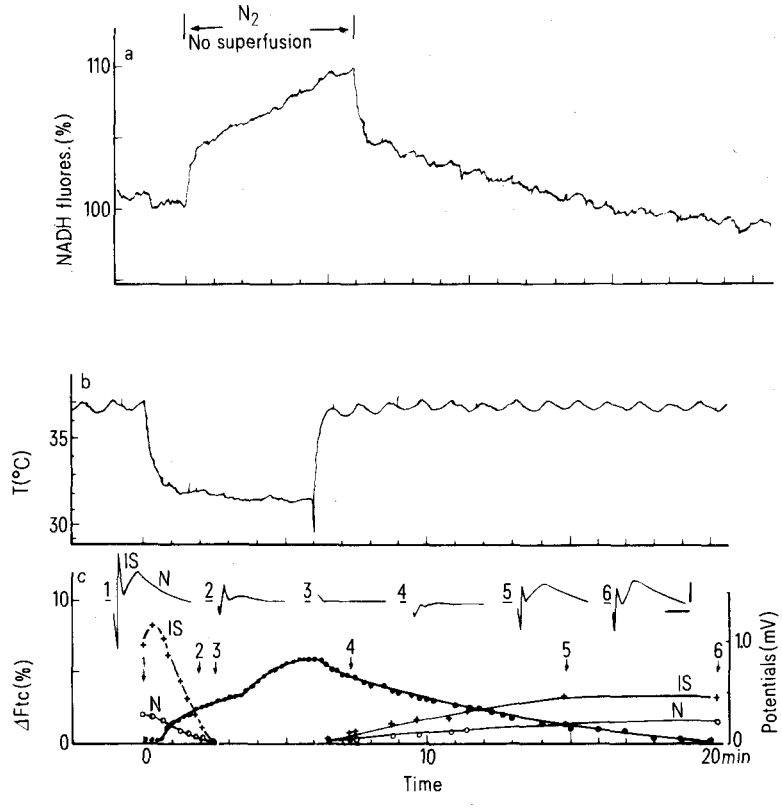


Fig. 3. *A* The NADH fluorescence changes of the olfactory cortical slice of the guinea-pig induced by stopping superfusion. The tissue was illuminated with the near-UV (360±50 nm) obtained from a water-cooled, 100-W Hg arc lamp filtered with Corning glass filter No. 5840. The blue fluorescence (450±50 nm) emitted from the brain slice was measured with a RCA 931B photomultiplier screened with 2 gelatin filters, Kodak Wratten No. 2C and No. 47. The output voltage of the photomultiplier was 800 V and the response time (99%) of the fluorometer was set at 3 sec. The fluorescence signal from the brain slice at 37 °C was arbitrarily chosen as 100%, and all fluorescence changes were calculated as percentages of this level. The anode current at the 100% fluorescence level was approximately 45 nA. *B* The temperature variations of the brain slice before, during and after the temporary stoppage of superfusion. *C* The temperature-corrected NADH fluorescence changes (ΔF_{tc}) calculated from figures 3, A and B, using figure 2 as the calibration curve. Arrows indicate the time points when the field potentials were photographed from the oscilloscope screen. Also shown are the time course of the IS (+) and N(o) potentials following cessation or resumption of superfusion. IS, initial spike (due to the olfactory tract potentials); N, negative potential (due to trans-synaptic depolarizations). Negative polarity upward. Calibration bars; horizontal = 5 msec and vertical = 0.2 mV.

Discussion. The present experiments demonstrate that the combination probe consisting of a micro-light guide[8,9] and a Ag-AgCl wire electrode is sensitive enough to measure hypoxia-induced changes in the tissue NADH fluorescence and field potentials from local areas (about 400 µm in diameter) of the guinea-pig olfactory cortical slice. When the superfusion of the brain slice was interrupted, a biphasic increase in fluorescence to the extent of about 10% of the basal fluorescence was observed. It was shown that approximately one half of this fluorescence increase was due to tissue anoxia and the other half due to the temperature decrease accompanying the stoppage of superfusion. The generation of potentials was found to be suppressed rather early in the hypoxic period, when the temperature-corrected NADH fluorescence increase was about half maximal. However, the recovery of potentials following the resumption of superfusion was concurrent with the decrease in the temperature-corrected NADH fluorescence. The kinetic correlation found between the oppositely changing field potentials and temperature-corrected tissue NADH fluorescence may be a consequence of the alterations in the intracellular ATP concentration caused by superfusion stop or resumption of superfusion. We have recently found that the pyridine nucleotide fluorescence increase observed in hemoglobin-free perfused rat liver during ischemia is inversely related to the ATP content of the liver tissue (S. Ji, J. Höper and M. Kessler, unpublished observation). Assuming a near-equilibrium relationship between pyridine and adenine nucleotide systems through the glyceraldehyde phosphate dehydrogenase and 3-phosphoglycerate kinase reactions, it was possible to account quantitatively for the fluorescence increase in terms of the decrease in tissue ATP content. Such a near-equilibrium relationship was also found in human erythrocytes[13], in liver freeze-clamped in vivo[14] and in suspensions of isolated hepatocytes[15]. Thus, it is reasonable to assume that pyridine and adenine nucleotide systems are in near-equilibrium in the olfactory cortical slice in the ischemic state and that an increase or a decrease in the pyridine nucleotide fluorescence of the cortical slice reflects a decrease or an increase of tissue ATP level, respectively. Finally, it must be pointed out that the potential changes observed here are not due to temperature changes in the cortical slice, since temperature changes induced by stopping or resuming superfusion are complete within about a minute, whereas potential changes occur over a much longer time period (figure 3, C).

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