Proton accumulation plays a role in the amplitude, but not in the time for the onset, of the vascular response to anodal current-induced vasodilatation

In the previously presented experiments, pH changes have been presented as a possible candidate either directly responsible for the excitation of nociceptors or rather acting as a sensitisor of the response to break excitation, thus explaining the increase in amplitude of the anodal vascular response with the duration of current application. Then, complementary protocols were designed to test alternative anionic and cationic buffers. We hypothesised that, as compared to deionised water, an alkaline buffer of the eventual proton accumulation (e.g. bicarbonate) would abolish, or at least decrease, the amplitude of the response to anodal current application, whereas an acid buffer or a neutral salt would not. Then, a new series of experiments was performed using four different vehicles: deionised water, sodium bicarbonate (250 mM in deionised water), sodium acetate (250 mM in deionised water) and sodium chloride (125 mM in deionised water) solutions. Experiments were performed with the same apparatus and experimental conditions as in the printed paper, on eight new subjects (4 males, 4 females; 28.5 ± 5.5 years, 61.6 ± 13.9, 168.4 ± 9.8 cm). In each subject, during a first experiment, 5 min anodal current applied through two of the four vehicles chosen randomly using two current suppliers. The cathodes were positioned on disposable Ag/AgCl adhesive electrodes, 5 cm from the iontophoresis patches. The two other vehicles were tested with the same procedure during a second experiment separated by at least 1 week from the first one. Subjects were not aware of which vehicle was used for current application.

RESULTS

No significant change was observed in the reference probe, arterial blood pressure and local skin temperature throughout the experiments. The subjective sensation to current application did not appear different to the subjects between sodium bicarbonate as compared to deionised water, or other vehicles, despite the wide difference in the vascular responses observed. Results for LDF_{rest} were not significantly different among the various solutions, respectively 6.9 ± 3.0, 7.6 ± 3.3, 7.5 ± 2.0 and 6.1 ± 3.4 %MVD for sodium acetate, sodium bicarbonate, sodium chloride and deionised water. Iontophoresis through sodium bicarbonate resulted in a significant decrease of the amplitude of the anodal current-induced vasodilatation: LDF_{peak} was 15.8 ± 14.3 %MVD for bicarbonate ($P < 0.05$ *vs.* all other solutions). On the other hand, vasodilatation was not significantly decreased with sodium acetate (49.8 ± 26.4 %MVD) and sodium chloride: (69.8 ± 40.9 %MVD) as compared to deionised water (65.8 ± 36.5 %MVD). Time for the onset of vasodilatation (T_{vd}), ranging from 6.15 to 6.25 min from current start for sodium acetate, sodium chloride and deionised water, was comparable to those reported in the printed paper for deionised water. With bicarbonate solution, T_{vd} as proposed in the printed paper could not be found in the experiments due to the lower LDF change rate resulting from current application. Nevertheless if T_{vd} was searched as the first of first of four consecutive 5 s intervals for which the derivative value of LDF was superior to the mean ± standard deviation (S.D.) instead of the mean ± 2 S.D. of the derivative values of the resting period, vasodilatation to anodal current application occurred at 6.15 min, then at the same time as those found in deionised water experiments or other solutions.

DISCUSSION

Some authors (Roth, 1994) relate the underlying mechanisms of anodal break excitation in nerves to the intrinsic properties of sodium channel kinetics. These properties may then depend on sodium concentration in the extra-cellular space. Using various sodium salt solutions could lead to homogeneous decrease of the response to current, and could be the underlying mechanism responsible for the decreased response to hyperosmolar solutions reported in previous experiments (Asberg *et al.* 1997). This was not the case in our experiments, and the attenuation of the amplitude of vasodilatation does not seem to be related to hyperosmolarity of the solutions and/or presence of sodium ions, since it was not found with the acid buffer sodium acetate, nor with sodium chloride.

It has been previously reported that the use of buffering salts rather than deionised vehicles to buffer pH changes decreases but does not abolish the response to the current (Sato *et al.* 1993; Berliner, 1997; Guffey *et al.* 1999). Consistent with these reports, these complementary data suggest that proton accumulation would play a major role in the mechanisms determining the amplitude of anodal vasodilatation in our experimental conditions, possibly through sensitisation of nociceptors to excitation. Inversely, it seems to have litte if any influence on the time for the onset for the vascular response. Thus, the delay for the vascular response at the anode seems independent of pH changes. The cause for the delay is still unsolved and will require further experiments. Finally, when performing experiments with anodal iontophoresis in water solutions, bicarbonate solutions should be preferred to deionised water or sodium chloride solutions to decrease the amplitude of the so-called 'non-specific' response to current application.