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INFLUENCE OF SYMPATHETIC NERVE STIMULATION ON FLOW VELOCITY IN PULPAL VESSELS

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Using a modification of the flying spot method for estimation of flow velocity in micro vessels, comparative observations were made on arterioles, venules and capillaries in the pulp of the rat incisor by vital microscopy. It was found that stimulation of the sympathetic nerves to the tooth, either at its apex or in the cervical sympathetic trunk, produced marked reduction of flow which was followed by a return to normal velocities at the cessation of stimulation. Direct stimulation of the sympathetic trunk produced very rapid reduction of flow velocity. Changes were also observed in response to administration of anesthetics and other procedures.

An abrupt reduction in the pulpal circulation of the canine tooth of cat has been observed when the cervical sympathetic nerve trunk was stimulated electrically (*Edwall & Scott*, 1971). Using the tracer disappearance method it was found that stimuli at frequencies between 3 and 10/sec could produce almost complete arrest of circulation within two minutes while lower frequencies resulted in comparably less pronounced effects. The magnitude of such circulatory reduction was far greater than that of the increase normally seen during warming the tooth.

The method of tracer disappearance depends not only on flow velocity in the network of fine vessels in the pulp near the site of the tracer depot, but also on the rate of diffusion from the depot to the capillaries and on capillary permeability. On the other hand, a more direct determination of velocity changes in fine vessels can be obtained by the method of vital microscopy using the preparation of the rat incisor as described by *Pohto* and *Scheinin* (1957, 1958) and *Scheinin* (1966). Direct observation has the

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further advantage of providing velocity information at intervals as short as 15 seconds or less, compared to 1 or 2 minutes for tracer disappearance, so that rapid changes can be more accurately recorded. Direct observation of vessels in the rat tooth also makes possible comparative measurements of flow changes in several categories of vessels under similar conditions of sympathetic stimulation. Finally, variations of flow due to turbulence and laminar flow gradients dependent on wall friction within a given vessel can be examined and quantitated by this method.

MATERIALS AND METHODS

The experimental animals were rats of the Sprague-Dawley strain. Operations were performed on a total of 23 animals from which 11 were selected for the complete experimental procedure. Following tracheostomy under intraperitoneal anesthesia (Nembutal®), the ligament connecting the two halves of the mandible was cut and one half was exposed. The alveolar bone covering the incisor was partially removed and the tooth was thinned down from the labial and from the lingual side. Preparation of the lingual side was continued so that the circulation could be observed through a thin dentinal layer usually 30—40 μm thick.

The completed preparation was mounted on a small table maintained at 37.6°C while the tooth was clamped in a horizontal position under the microscope objective, and lightly covered with eugenol. In the present experiments a modification of the flying spot technique of *Brånemark* (1959) has been employed in which the single visual tracer seen in the microscope was replaced by a moving train of multiple spost. The present method served to average the velocities of a population of cells moving within the vessel and did not depend on the movement of only a single cell as in the double slit method of *Wayland & Johnson* (1967). Previous experience has shown that single cell velocity measurements are distorted by turbulence in the vessels under observation whereas the present procedure averaged out such minor fluctuations. The special spotrain generator (designed by Mr. M. Fugita in the Instrumentation Laboratory of the Institute of Neurological Sciences at the University of Pennsylvania) was connected to the oscilloscope so that a moving train of eight, equally spaced bright spots appeared continuously and their velocity could be controlled by a calibrated remote control. These spots were then observed in the field of the microscope through a half silvered prism and their axis of travel was adjusted to that of the vessel under observation by rotation of a dove

prism in the optical system. It was then possible to easily adjust the translational speed of the spot train on the face of the oscilloscope to coincide with that of the erythrocytes in the vessel and read off from the control box their flow velocity in micrometers/second (μm/sec.).

Initial observations on the effect of sympathetic stimulation on flow velocity in pulp vasculature were obtained by insertion of a fine coaxial needle electrode through the soft tissues roughly parallel to the major axis of the tooth so that its uninsulated tip was close to the apex. While such stimulation was largely restricted to the nerves entering one tooth, both efferent and afferent pathways were excited and, in some placements, spread of the stimulus to neighboring muscles was evident. In all instances where persistant motor activity was observed, the results were discarded since it was found that such muscular contraction in the vicinity of the apex of the tooth frequently resulted in increased flow rates during stimulation. To prevent cumulative effects, all nerve stimulations were separated by resting periods of 20 minutes and successive periods of stimulation were only attempted when the initial control velocity had shown no significant change compared to previous values.

The course of the sympathetic trunk in the rat runs roughly parallel to the trachea but in a more dorsal position: its exact relation to adjacent tissues being inconstant. At this level it may run in (a) the same sheath as the vagus nerve, or (b) in the same sheath as the carotid artery or (c) in some path unrelated to either of these structures. To expose the nerve, the previously prepared tooth surface was suitably protected from cooling and drying and the animal repositioned on its back. The skin incision for exposing the trachea was lengthened and by careful blunt dissection of muscles and indigenous vessels the cervical sympathetic trunk was exposed, ligated, and sectioned as far caudal as possible (Fig. 1). The exposed length of nerve was freed from adherent tissues for a distance of about 2 cm. and then carefully replaced under neighboring tissues to protect it until it was needed for stimulation. Correct identification of the sympathetic trunk was tested by observation of eye protrusion during stimulation of the cut nerve.

In those experiments where stimulation was applied directly to the previously dissected sympathetic nerve trunk, the adjacent tissues were retracted to allow placement of the nerve on platinum wire electrodes, connected to a Tektronix type 161 pulse generator. The nerve and electrodes were then covered with Plastibase® (Squibb) to prevent drying or current leakage (Fig. 1). Since the rat was initially lying on its right side for clamping the tooth beneath the microscope, the introduction of the electrodes required no change in position. The stimulating voltage necessary for a

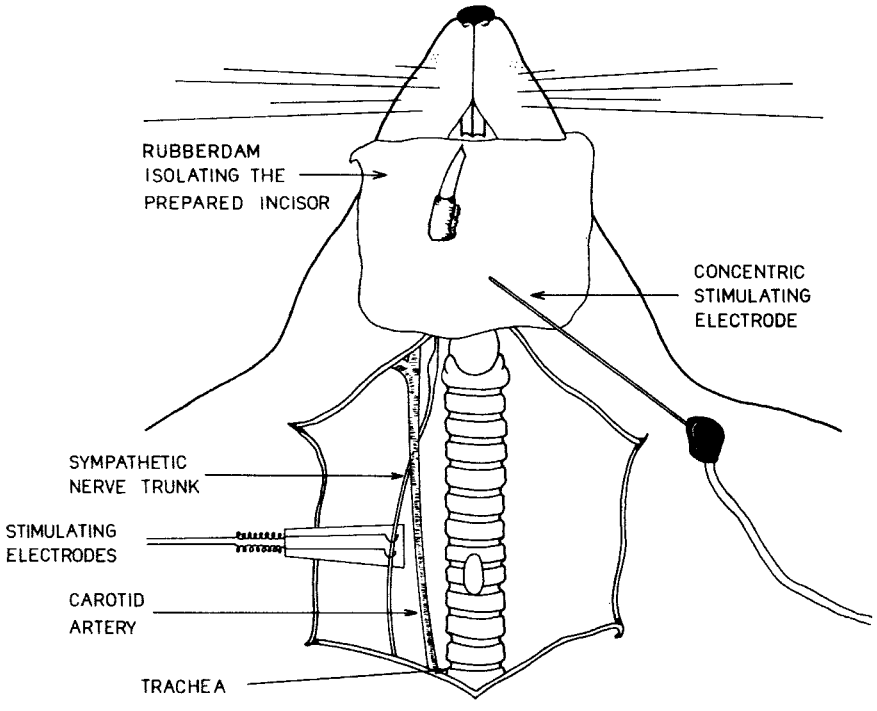

Fig. 1. Ventral view of rat head and thorax. Approximate point of insertion of concentric stimulating electrode. Alternatives stimulation by exposure of sympathetic nerve trunk in vicinity of carotid artery (as shown) and mounting of platinum stimulating electrodes.

maximal response was considerably lower with the isolated nerve than with apical stimulation due to the lower impedance of the platinum electrodes when compared to the coaxial electrode.

RESULTS

Stimulation of nerve fibers at the apex of the incisor

Normal flow velocity measurements were most readily obtained in venules and capillaries: the usual high velocity in arterioles making difficult the accurate visual synchronization between the movement of the erythrocytes and the moving train of light spots. During nerve stimulation a marked reduction of flow rate was observed in all classes of vessels: the magnitude of the effect being related to the frequency and the voltage of the stimulus applied. The maximal effect observed in a large venule (40 μm. diameter)

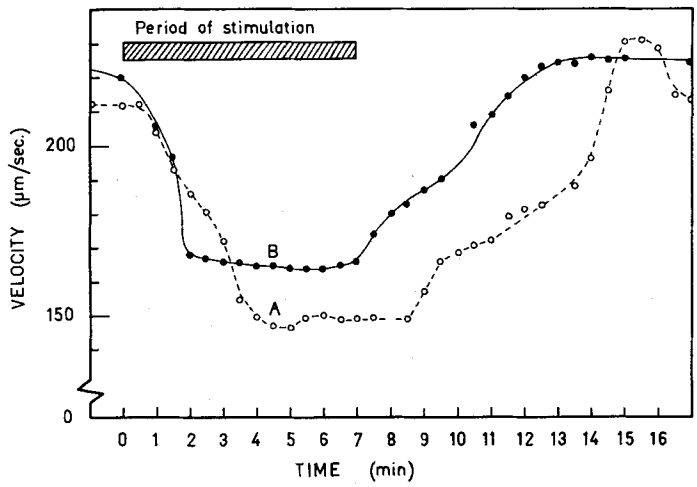

Fig. 2. Changes in flow velocity of large venule (40 μ) produced by electrical stimulation at apex of tooth by concentric needle electrode. A) Stimulation maximal 10/sec., 20 v., 1 msec.; B) Stimulation submaximal 10/sec., 15 v., 1 msec.

was obtained using stimuli of 10/sec., 20 v., and 1 msec. duration (Fig. 2, A). During the first four minutes of stimulation the initial velocity of 215 μm/sec. declined progressively until a value of 145 μm/sec. was reached (38 % reduction). During the remaining 3 minutes of stimulation no further significant change was observed. Recovery of flow velocity to its initial value required ten minutes after the end of stimulation. The form of the recovery curve often consisted of two roughly sigmoid phases, suggesting the possibility of release from more than one constrictor site.

The result of sub-maximal stimulation using 15 v., 10/sec. is shown in Fig. 2, B. While the resulting change in velocity followed a similar course, it only decreased to 165 μm/sec. (32 % reduction). This sub-maximal stimulation failed to produce a velocity exceeding the initial control during the later recovery period although such »overshoot» was commonly observed when maximal stimuli were applied. The final velocity was within 1 % of the initial resting value and this degree of recovery was found in most experiments.

Application of maximal stimulation under identical conditions also produced a reduction of flow velocity in small venules (15 μm diameter). In a typical vessel the initial velocity of 143 μm/sec. was reduced by 36 % after four minutes stimulation. This recovered to within 1 % of its initial value six minutes after the end of stimulation, having first passed through a period

of supranormal flow velocity. Thus, the pattern of flow reduction did not show significant differences when compared in vessels of differing diameter, although the initial flow rate varied considerably.

A higher initial flow velocity (mean 880 μm/sec.) was found in arterioles (20 to 40 μm in diameter) and a somewhat greater percentile reduction (48 %) was observed during nerve stimulation. In other respects the response to stimulation resembled that observed in venules. Changes in the flow rate of capillaries were less consistant since cessation of flow was often observed, either due to the effect of nerve stimulation on the overall vascular bed or due to the shunting of flow into other channels. However, except for periods of such standstill, the velocity changes during nerve stimulation were similar to those seen in larger vessels. Initial velocities in capillaries were observed as high as half that in small venules but often were considerably lower. The recovery of normal flow rate was not found to be delayed in capillaries more than in venules.

Stimulation of sympathetic nerve trunk

Most observations of low velocity during direct sympathetic nerve stimulation were made on venules because of the (a) greater accuracy of measurement and (b) the large variation in vessel diameter available in a single preparation. The reduction in flow velocity observed with direct stimulation of the cervical sympathetic nerve trunk was always far greater than that obtained using stimulation at the incisor apex with coaxial electrodes, and the resulting changes occurred much more rapidly. For example, cessation of flow was observed in one small venule after only 11 seconds of nerve stimulation, but in most instances the maximum slowing was seen in 20--40 seconds. The rate of recovery was also much more rapid, reaching the initial velocity in less than 1.5 minutes after the end of stimulation. This was often followed by a period of supra-normal velocity lasting up to four minutes (Fig. 3). In many of the experiments of this series, a drift in resting flow velocity was observed and this appeared to be related to the basal systemic activity of the animal as described below.

The effect of varying stimulus strength was observed in several examples from a single experiment. When stimulus to the sympathetic nerve was 2.2 volts at 6/sec. the flow velocity reduction in a small venule was 40 % in 2 minutes while a stimulus of 6.6 volts at the same frequency produced a 71 % velocity reduction in the same vessel. These results were quantitatively

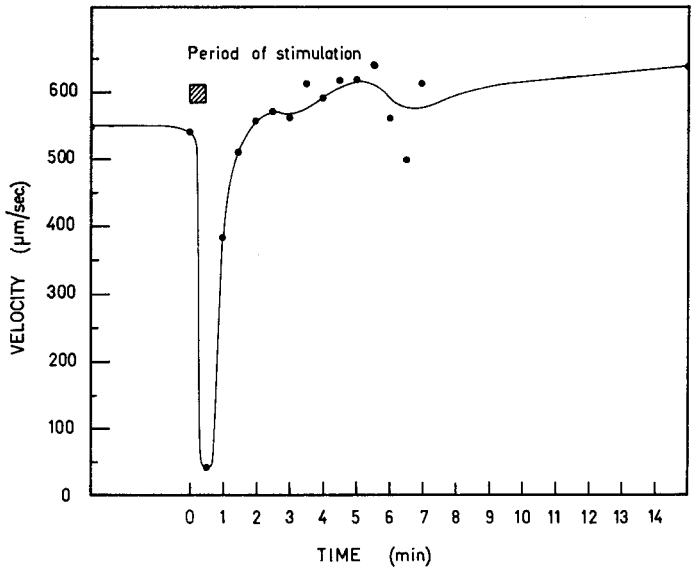

Fig. 3. Changes in flow velocity of large venule produced by electrical stimulation of sympathetic nerve trunk using 3/sec., 6 v., and 5 msec.

reproduceable provided that an interval of 20 minutes elapsed between periods of stimulation.

The effect of altering stimulus frequency was more variable and less pronounced. Using a stimulus of 6 volts and observing a small venule, frequency was varied from 1.0 to 6.0/sec. The resulting reductions in velocity varied from 66 % to 100 %, which were more dependent on the basal activity and anesthetic level of the animal than on the frequency of stimulation. This suggests that the frequencies chosen were all close to maximally effective and that frequencies below 1.0/sec. would probably also have produced substantial reductions of flow velocity. In general it was found that when either frequency or stimulating voltage was increased the effect was to make the initial reduction in flow velocity more rapid and more complete while the recovery tended to be slower.

The effect of any given level of stimulation was found to be dependent on the diameter of the venule in which it was observed. Venules were classed as large (40—60 μm.) or small (25—40 μm.) The mean initial velocity for the series of large vessels observed was 420 μm/sec. while that for the small vessels was 215 μm/sec. When the same intensity of sympathetic stimulation was applied the percentile flow retardation was consistently greater in small

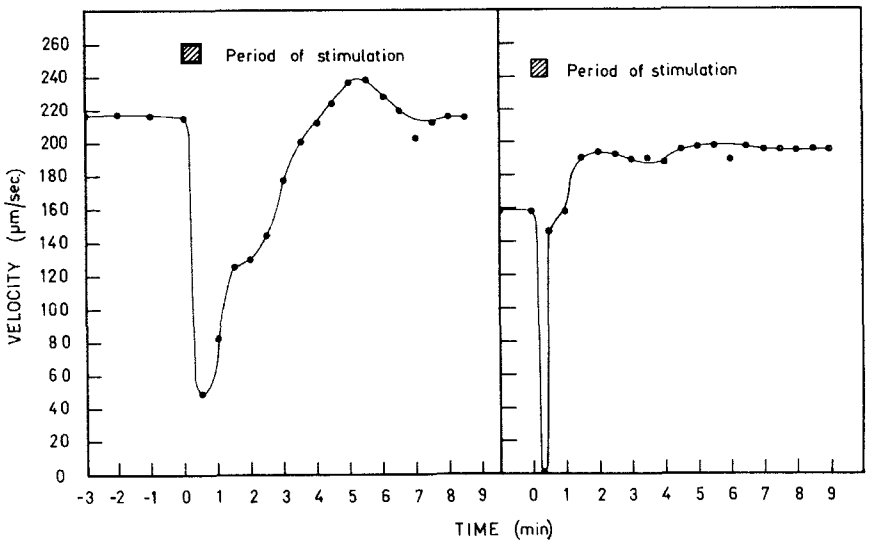

Fig. 4. Changes in flow velocity in small venule produced by electrical stimulation of sympathetic nerve trunk using 3/sec., 6 v., and 1 msec. a) Stimulation 30 seconds duration just prior to anesthetic supplement and more than 2 hrs since previous supplement. b) Stimulation 20 seconds duration 30 minutes after anesthetic supplement which immediately followed record a).

vessels than large. For example, at a time when a stimulus of 6.6 v. 6/sec. produced a 71 % velocity reduction in a small venule, the same stimulus produced only a 25 % reduction in a near by large venule. Although the percentile reduction in flow velocity was influenced by the depth of anesthesia (see below) the relative effect of a given level of stimulation on small and large venules was much greater on the former as in the example above.

Comparisons of the experimental data on arterioles (which was limited due to difficulties in obtaining accurate measurements of high flow velocities) with that on venules showed that with similar nerve stimulation flow velocity in the former was much less reduced than in the latter. Thus, in comparison with the above data from venules, it was found that the flow velocity in a small arteriole was reduced by 50 % when a 6 v. 6/sec. stimulus was applied to the nerve. An even greater difference was found when a sub-maximal stimulus of 2 v. 6/sec. was applied in which case the flow velocity in the arteriole was only reduced by 20 % while that in the adjacent main venule was reduced by 100 % to standstill. No strength of stimulus was found which would produce stoppage of flow in arterioles.

The initial resting flow velocity in any given vessel was found to be markedly influenced by the level of anesthesia as determined by the time elapsed since the last administration of anesthetic to the animal. The initial anesthesia required a supplement every 60 to 90 minutes during periods of recording and the effect of these supplements on flow velocity in a small venule can be seen in Fig. 4. In Fig. 4a the animal was in light anesthesia and occasionally showed traces of motor activity. One minute after the end of this recording, Nembutal, 0.05 ml (2.5 mg) was injected intraperitoneally and 29 minutes later the record shown in Fig. 4b was started; the nerve stimulation being 30 minutes after the injection. Stimulation in both instances was 6 v. and 3/sec. Comparison of these records shows that increasing the level of anesthetic reduced the resting flow velocity and enhanced the effect of sympathetic nerve stimulation so that flow velocity falled to zero in the first 30 seconds. These effects have been seen as early as 4 minutes after supplemental anesthetic injection and gradually disappeared over a period of an hour.

DISCUSSION

The effect of stimulation by concentric needle electrode in the apical region of the tooth has been compared to stimulation of the isolated cervical sympathetic nerve and has been shown that the same general type of reduction in flow velocity was obtained, but use of the latter technique produced more rapid changes and much higher percentile flow reduction. Although these dramatic effects were obtained with lower stimulating voltages, the difference might have been partially due to differences in electrode impedance so that the stimulating current flow could have been similar in both instances. However, maximal stimulation of the excised sympathetic trunk must have excited all but a few nerve fibers, thus causing major neurogenic vasoconstrictor activity, while stimuli applied to the apical region of the tooth were of uncertain effectiveness since the tip of the concentric needle could only be placed in approximate relation to the fine nerve bundle supplying the tooth and were limited in amplitude by interference from excitation of neighboring skeletal muscle.

The time required for maximum flow reduction was markedly different with the two types of stimulation; excitation of the sympathetic nerve trunk producing much the faster responses. It seems probably that this was also due to the greater effectiveness of direct stimulation of sympathetic nerve in which case all constrictor sites were presumably activated. The fact that recovery of flow velocity also showed a similar difference in rate depending

on the site of stimulation suggests that it was probably the number of constrictor sites affected rather than the degree to which each contracted that was responsible.

In almost all experiments, the flow velocity following nerve stimulation showed a rapid phase followed by a slow phase of recovery. The stronger and more effective the stimulus, the more complete was the rapid phase of recovery (Fig. 4b) while submaximal stimuli showed only a small rapid recovery followed by a prolonged slow return to the initial flow velocity (Fig. 2b). These observations would be consistant with other evidence (*Abboud*, 1972) suggesting a proximal neurogenic constrictor site (possibly close to the apex of the tooth) and a more distant site possibly activated by a humoral agent.

Results from the previous study using the method of tracer disappearance (*Edwall & Scott*, 1971) showed a marked dependance of sensory unit excitability on the local circulation. While simultaneous electrical recording from afferent neurons was not possible in the present experiments, the results reported above support the concept that stimulation of the sympathetic pathway produces a prompt fall in flow velocity and this would be consistent with the changes in receptor excitability which were found in the earlier study. It, thus, seems probable that the sensation of pain of intradental origin from teeth may be strongly influenced by the sympathetic nerve control of blood flow in the pulp and that such influence may cause rapid changes in sensitivity.

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