Br. J. Pharmac. (1972), **46**, 676–687.

The effect of bradykinin on denervated tongue

B. RADMANOVIĆ* AND SONIA H. TORRES†

From the Department of Physiology, University of Birmingham, Birmingham B15 2TJ

Summary

1. The contractile response of the chronically denervated tongue of the cat to chorda stimulation, and to close arterial injections of bradykinin, acetylcholine (ACh) and other drugs was examined.

2. Bradykinin in doses of 50 ng–20 μg injected close arterially always produced a contractile response of the denervated tongue. Sodium nitrite (1 mg i.a.) and isoprenaline (3–200 ng i.a.) also produced contracture; histamine (40–100 ng i.a.) evoked an increase in tension in only 2 out of 5 experiments.

3. Tubocurarine in doses of 0·25–1 mg injected intra-arterially, produced a large and long-lasting contracture of the denervated tongue. When the contracture was over, the effect of bradykinin was reduced to about half; the effects of ACh and chordo-lingual nerve stimulation were markedly reduced (over 80%), and those of sodium nitrite and isoprenaline were transiently abolished. Gallamine only slightly reduced the effect of bradykinin.

4. Close intra-arterial injection of physostigmine (100 μg) potentiated the effect of ACh and chordo-lingual nerve stimulation, but did not increase the response to bradykinin.

5. Cocaine (1 mg/kg i.v.) deeply depressed the response to bradykinin, and moderately reduced the responses to ACh (41%) and to chorda stimulation (66%).

6. In 2 out of 7 experiments, close arterial injections of bradykinin (100–500 ng) to the denervated tibialis anterior muscle of the cat, produced a contractile response. Bradykinin in small doses (200–250 ng) injected immediately before ACh potentiated its effect. On the other hand, the effect of ACh was depressed when given immediately after a big dose of bradykinin (10–15 μg).

7. The possible mechanism of action of bradykinin and other substances on denervated muscle is discussed.

Introduction

The muscles of the tongue, as other striated muscles, become supersensitive to acetylcholine (ACh) after denervation. Frank, Nothman & Hirsh-Kauffmann (1922) reported that 4 days after motor nerve section, intravenous injections of ACh produced contracture of the muscles of the tongue. Emmelin & Nordenfeldt (1959) measured the increase in sensitivity in the tongue of the cat and found that it was 100–2,500-fold. A sign of the denervation supersensitivity is the long known 'Philipeaux–Vulpian phenomenon', which is the contracture of the tongue produced

* Present address: Facuity of Medicine, Department of Pharmacology, Beograd 7, Yugoslavia.
† Present address: Escuela de Medicina, Departamento de Ciencias Fisiológicas, Universidad de Oriente, Ciudad Bolívar, Venezuela.

by stimulation of the chordo-lingual nerve, a few days after the section of the hypoglossal nerve (Philipeaux & Vulpian, 1863). Dale & Gaddum (1930) suggested that the contracture was produced by ACh liberated at the parasympathetic nerve endings.

The response of denervated muscle to ACh has been thoroughly investigated (Brown, 1937; Rosenblueth & Luco, 1937; Ginetsinsky & Shamarina, 1942; Axelsson & Thesleff, 1959; Miledi, 1960; Albuquerque & Thesleff, 1968; Feltz & Mallart, 1971). The effect of other substances, especially those that do not have any action on normally innervated striated muscle, has been studied less. In 1946, Ado, Ginetsinsky & Shamarina found that denervation of the tongue in the dog increased its sensitivity, making it responsive to arecoline; but if denervation was accompanied by sensitization to a foreign protein, the range of substances to which the muscle reacted was widely increased; cholinomimetic drugs like physostigmine and pilocarpine; sympathomimetic drugs like adrenaline, ephedrine and benzedrine; histamine, morphine and even substances like curare and atropine were able to elicit a contractile response of the muscles of the tongue, when injected into the artery. Responses of the denervated diaphragm to bradykinin, histamine and 5-hydroxytryptamine were shown by Alonso De Florida, Del Castillo, González & Sánchez (1965a, b) both in sensitized and non-sensitized guinea-pigs. On the other hand, Bhoola & Schachter (1961) found no response to bradykinin, histamine or pilocarpine in denervated rat diaphragm.

The present paper deals with the response of the denervated muscles of the tongue of the cat to close arterial injections of bradykinin and other vasodilator substances. The effect of bradykinin is compared with the effect of exogenous ACh, similarly injected, and with the endogenous liberation of ACh produced by stimulation of the chordo-lingual nerve. The action of bradykinin on other chronically denervated muscles was also examined.

Methods

Experiments on the tongue

The experiments were performed on 32 cats of either sex weighing 1·5–3·25 kg. The nerve sections were all carried out under pentobarbitone anaesthesia (35 mg/kg) and with full aseptic precautions, 6–9 days before the final experiment. The hypoglossal nerve was freed on the right side of the neck and 1–2 cm excised.

In the final experiments the cats were anaesthetized with chloralose (70–100 mg/kg) after induction with ethyl chloride and ether, or with pentobarbitone (35 mg/kg). For close arterial injection of drugs, the external carotid artery on the denervated side was cannulated retrogradely, rostral to the origin of the submaxillary artery. All the branches of the external carotid except the lingual artery were tied. Transmission to the tongue of the respiratory movements of the larynx was minimized by passing a large, curved needle through the larynx in the region of the hyoid bone, and fixing its two ends to vertical supports attached to the operating table. The mouth of the cat was held open and the tip of the tongue was connected to a steel spring with a thread. A silicon crystal strain gauge on the spring was connected via a Wheatstone bridge to a Devices recorder. Sometimes a Grass FT.03 force displacement transducer connected to a 7P polygraph was used. The chordo-lingual nerve was cut and its distal end fixed in a fluid electrode. In some experi-

ments the hypoglossal nerve on the contralateral side was also prepared for stimulation in the same way. The chordo-lingual nerve was stimulated by square wave impulses of 1 ms duration at 30 Hz and intensity between 8 and 15 V. The impulses used for the hypoglossal nerve were of 0·24–0·6 V, the other parameters being the same.

Experiments on tibialis anterior

In 7 cats, about 1 cm of the lateral popliteal nerve was excised under pentobarbitone anaesthesia and full aseptic conditions, one week before the final experiment. To record muscle tension, the tibia of the denervated side was drilled and fixed to metal supports holding the leg rigidly in a semi-flexed position ; the tendon of tibialis anterior was dissected free and attached to a strain gauge for tension recording. The anterior tibial artery was cannulated for close retrograde injections into the muscle.

Experiments on soleus

In 3 cats the soleus muscle was denervated under pentobarbitone anaesthesia and full aseptic conditions by cutting the sciatic nerve high up in the thigh. In the final experiments, one week later, gastrocnemius and plantaris muscles from the denervated side were removed and the soleus tendon was freed and attached to a strain gauge. The animal was laid prone and the leg was fixed as above, semi-flexed. For close arterial injections a cannula was introduced into the proximal end of the ligated anterior tibial artery and passed centrally up the vessel to the point at which the posterior tibial artery leaves the main vessel. At the moment of injection the main artery was occluded in order to avoid retrograde flow of the drug.

In all experiments, arterial blood pressure was recorded from a femoral artery, using a Bell & Howell transducer.

Drugs were injected intra-arterially on the denervated side in volumes of 0·1–0·3 ml. The interval between injections was 5 minutes. Drugs used were: acetylcholine chloride (' Acécoline ', Lematte et Boinot), synthetic bradykinin (Sandoz, Parke Davis and Calbiochem), gallamine triethiodide (' Flaxedil ', May & Baker Ltd.), physostigmine sulphate (Hopkin & Williams, Ltd.), histamine acid phosphate (Fisher Scientific Company), (±)-isoprenaline sulphate (' Neo-epinine ', Burroughs Wellcome & Co.), sodium nitrite (Analar and BDH), cocaine chlorhydrate (Merck) and (+)-tubocurarine chloride (' Tubarine ', Burroughs Wellcome & Co.). Doses of bradykinin and physostigmine are expressed as base, all the others as salts.

Results

Effect of bradykinin, acetylcholine and chorda stimulation

In 31 experiments out of 32, close arterial injections of bradykinin to the denervated tongue produced a contractile response. This was seen as a visible contracture immediately after the injection. The amount of bradykinin required to produce an increase in tension of at least 1 g varied from 50 ng to 20 μg. In most experiments, the maximal response was obtained with 250 ng bradykinin. From the recordings of tension developed by the tongue, a fast rise and fall of tension was usually observed, as illustrated in Fig. 1A. In six experiments this response was

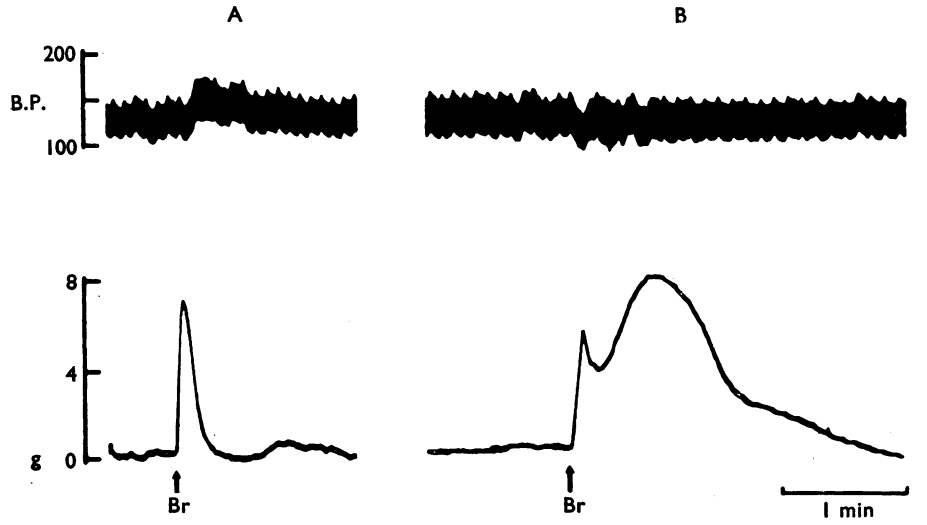

FIG. 1. Cat; chloralose. Upper record: arterial blood pressure. Lower record: tension of the tongue. At arrows (Br), 250 ng bradykinin was injected close arterially. Records A and B are taken from different experiments.

followed by a second, slow rise of tension (Fig. 1B). In seven experiments, only the slow response was seen.

In all experiments, a comparison was made with the response to close arterial injections of ACh. It was found that these preparations responded to very small doses of ACh: 0·1–10 ng produced a rise in tension of at least 1 g. The characteristics of the response to ACh were similar to those obtained with bradykinin and a dose of ACh that produced a similar response to that obtained with bradykinin could be found in each experiment. If higher doses of ACh were used, the height of the fast response increased, whereas that of the slow rise and fall of tension diminished. If higher doses of bradykinin were injected, no response was obtained. The contraction of the tongue produced by 250 ng bradykinin was usually of similar magnitude to that produced by 2 ng ACh. An injection of bradykinin did not modify the effect of a subsequent injection of ACh.

The chordo-lingual nerve was stimulated for 20–30 s with pulses of 1 ms duration, at 30 Hz. With 8–15 V a maximal response was obtained; the height of the contracture was similar to that obtained with 250 ng bradykinin.

Effect of tubocurarine and gallamine on responses to bradykinin, acetylcholine and chorda stimulation

It is generally accepted that tubocurarine competes with ACh for combination with receptor sites, and thus prevents the action of the transmitter. If this competition were specific, tubocurarine would not block the effect of bradykinin.

In 6 experiments, tubocurarine was injected intra-arterially to the tongue in doses of 0·25–1 mg. These doses were chosen because they diminished the contracture produced by stimulation of the contralateral hypoglossal nerve without producing a complete block. In every experiment tubocurarine itself produced a large contracture of the tongue lasting 1–20 min (Fig. 2), depending to some extent on the dose

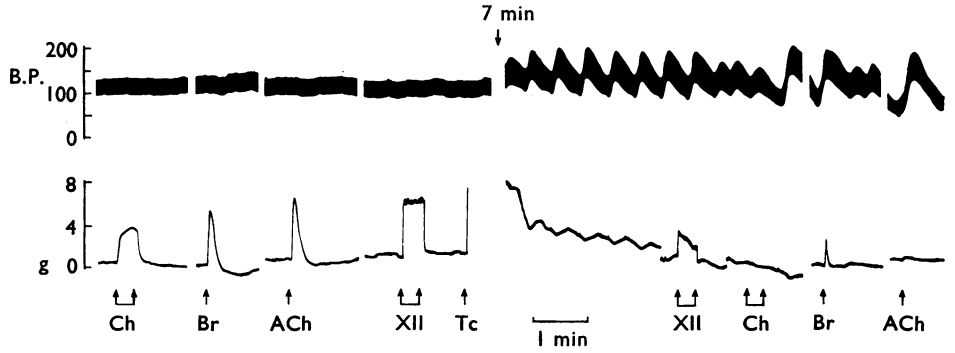

FIG. 2. Cat; chloralose. Upper record: arterial blood pressure. Lower record: tension of the tongue. At (Ch) and (XII), chordo-lingual nerve and hypoglossal nerve were stimulated. At (Br), (ACh) and (Tc), bradykinin 200 ng, acetylcholine 2 ng and tubocurarine 0·5 mg, were injected close arterially.

injected. Further injections of the same dose of tubocurarine produced either no contractile response, or a contracture of decreased force and duration. This effect has been described for other denervated muscles (McIntyre, King & Dunn, 1945; Sánchez & Luco, 1956; Bowman & Raper, 1964).

When the contracture produced by the initial dose of curare was over, the effects of both ACh injection and stimulation of the chordo-lingual nerve were greatly depressed or abolished (Figs. 2 and 3). Usually no recovery was seen at the end of 1 hour. The effect of bradykinin was reduced by about 56% and, in 3 experiments, returned to the control level within 1 hour.

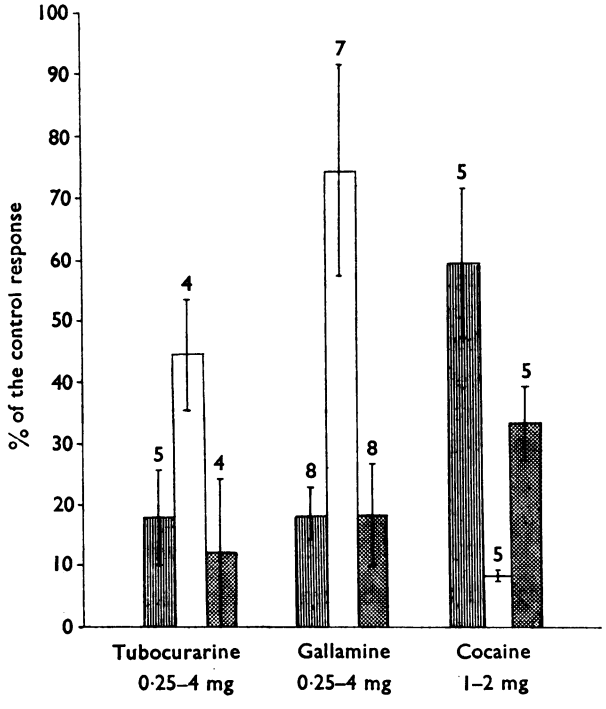

FIG. 3. Effect of tubocurarine, gallamine and cocaine on the contractile response of the denervated tongue to acetylcholine and bradykinin injections, and to chordo-lingual nerve stimulation. Control responses are expressed as 100 per cent. Columns show the mean values for the number of experiments indicated in the figures. Dashed columns, response to ACh; open columns, response to bradykinin; dotted columns, response to chordo-lingual nerve stimulation. Vertical lines give the standard errors.

The results of one experiment are illustrated in Fig. 2 ; 0·5 mg tubocurarine injected into the tongue produced a contracture lasting 7 minutes. Immediately afterwards, the contracture produced by stimulation of the contralateral hypoglossal nerve was reduced to one-third of the control response ; ACh produced a very small response ; the response to bradykinin was reduced to about one-half and the effect of chorda stimulation was abolished.

Gallamine, in doses of 0·25–4 mg injected intra-arterially also produced a contracture of the denervated tongue, but this was not so large or as long-lasting as that produced by tubocurarine. As with tubocurarine, the responses to ACh injection and to stimulation of the chordo-lingual nerve were greatly reduced or totally blocked. However, differences were noted in the recovery of the responses ; the contracture produced by nerve stimulation was restored to its previous size within 30 min, whereas the response to ACh injection was still depressed. The response to bradykinin remained unchanged in 4 experiments and was reduced in 3 experiments (see Fig. 3).

Effect of physostigmine on responses to bradykinin, acetylcholine and chorda stimulation

In order to exclude the possibility that bradykinin might be acting through release of ACh the effect of an anticholinesterase, physostigmine, was investigated. A dose of 100 μg was injected close intra-arterially into the denervated tongue. The response to chordo-lingual nerve stimulation, ACh and bradykinin injections was tested before physostigmine administration and 10–30 min afterwards. The response to ACh was always potentiated, increasing in duration and height ; in some experiments the response to ACh was abolished after a period of augmented response to this drug. The contracture produced by chordo-lingual nerve stimulation was increased and prolonged in 3 experiments and unchanged in one experiment. The response to bradykinin did not increase after physostigmine. The results of two different experiments are shown in Fig. 4. In A, the response to ACh was greatly enhanced while the response to chorda and bradykinin did not change. In B, the response to nerve stimulation was increased very much, that to ACh increased mainly in duration, and that to bradykinin showed some change in shape but did not increase. The injection of bradykinin sometimes produced movements of deglution which were transmitted to the tongue ; however, they could be differentiated from the underlying contracture.

Effect of cocaine on responses to bradykinin, acetylcholine and chorda stimulation

It has been demonstrated (Trendelenburg, 1966) that cocaine reduces the response of the cervical sympathetic ganglion to bradykinin without reducing ganglionic transmission ; therefore, cocaine was used to see whether the effect of bradykinin was due to stimulation of the intralingual ganglia. Cocaine was injected i.v. in doses of 1 mg/kg. Responses to chordo-lingual nerve stimulation, ACh and bradykinin injections were tested before and 10 min after injection of cocaine. In some experiments an additional dose of 1 mg/kg was administered totalling 2 mg/kg, and the responses were tested again. As shown in Fig. 3, the response to bradykinin was reduced by 92% ; however, the responses to ACh and nerve stimulation were unexpectedly also reduced by 41% and by 66% respectively.

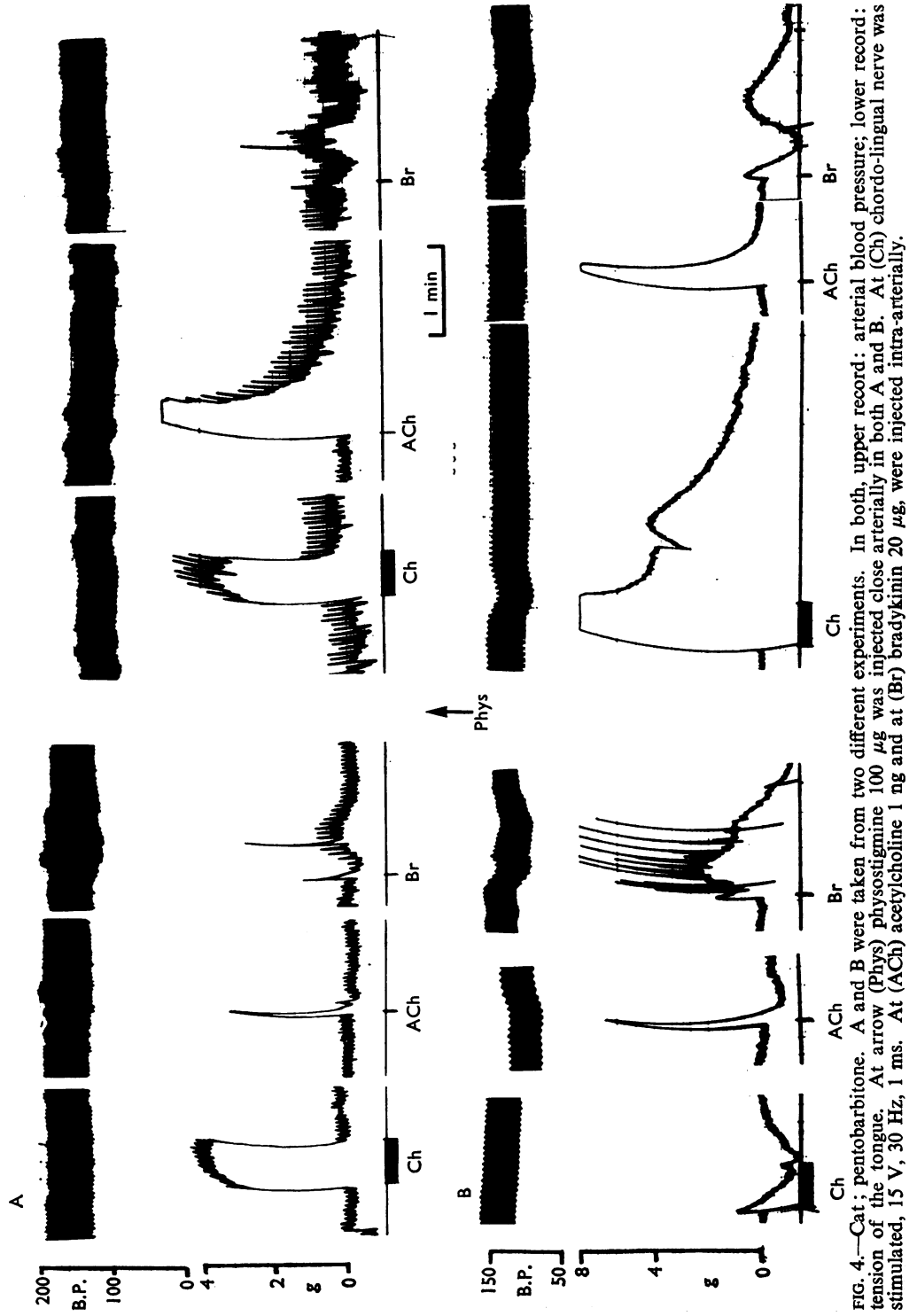

FIG. 4.—Cat; pentobarbitone. A and B were taken from two different experiments. In both, upper record: arterial blood pressure; lower record: tension of the tongue. At arrow (Phys) physostigmine 100 μg was injected close arterially in both A and B. At (Ch) chordo-lingual nerve was stimulated, 15 V, 30 Hz, 1 ms. At (ACh) acetylcholine 1 ng and at (Br) bradykinin 20 μg, were injected intra-arterially.

Effect of other substances that relax vascular smooth muscle

Other substances that relax vascular smooth muscle were tested on the denervated tongue to see if they would also produce a contractile response. Sodium nitrite, injected in doses of 1 mg, caused a large contracture of the denervated tongue, sometimes in the form of a two-phased response and occasionally a fast response. These effects were abolished by an intra-arterial injection of tubocurarine. As shown in Fig. 5, there was no response immediately after the contracture produced by tubo-

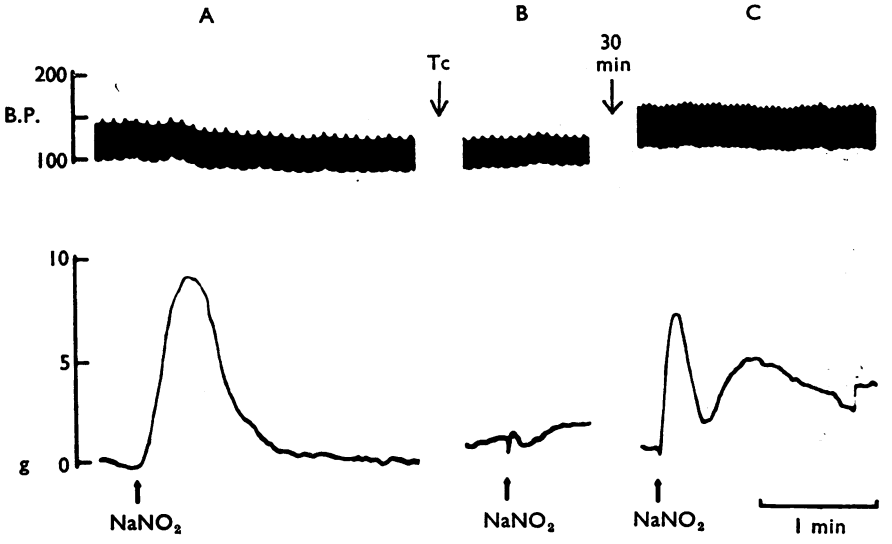

FIG. 5. Cat; chloralose. Upper record: arterial blood pressure. Lower record: tension of the tongue. At arrows ($NaNO_2$) 1 mg sodium nitrite and at (Tc) 0·25 mg tubocurarine were injected close arterially. Record B was taken immediately after the contracture produced by tubocurarine had subsided and record C, 30 min after B.

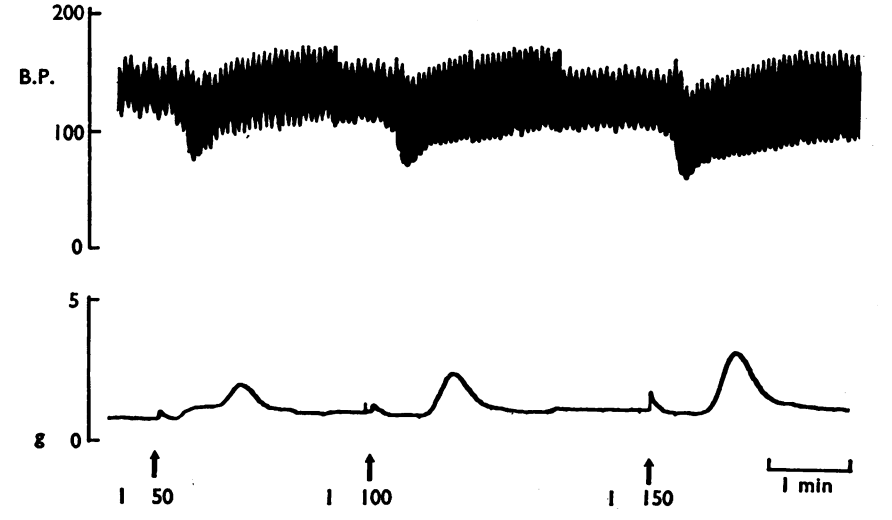

FIG. 6. Cat; chloralose. Upper record: arterial blood pressure. Lower record: tension of the tongue. At arrows (I) isoprenaline was injected close arterially in doses of 50, 100 and 150 ng.

curarine had subsided, but after 30 min, there was a response to sodium nitrite, though of a different shape.

Close arterial injection of isoprenaline, in doses from 3 to 200 ng regularly produced contracture, the actual effect being dose-dependent, as illustrated in Fig. 6, where responses to 50, 100 and 150 ng isoprenaline are shown. The effect of isoprenaline was transiently abolished by tubocurarine. Histamine, 40–100 ng, produced contracture in only 2 out of 5 experiments.

Effect of bradykinin on denervated tibialis anterior and soleus muscles

In view of the positive results obtained on the denervated tongue in response to bradykinin, the effects of this peptide on denervated skeletal muscle were also tested. The fast tibialis anterior and the slow soleus muscles were chosen.

Bradykinin (100–500 ng) produced a contractile response of the tibialis anterior muscle in only 2 out of 7 experiments. The sensitivity to ACh was also tested in these 2 experiments: in one, the muscle responded to a dose of 2 ng and in the other to 100 ng. In the other 5 experiments larger doses of ACh (200 ng–4 μg) were needed to produce a contractile response.

In the experiments in which bradykinin did not produce a rise in tension of the denervated tibialis anterior, it potentiated the effect of ACh ; doses of 200–250 ng injected immediately before 2–4 μg ACh, increased its effect 2–10 times. On the other hand, when the same doses of ACh were injected immediately after a big dose of bradykinin (10–15 μg) the effect of ACh was reduced to one-third or

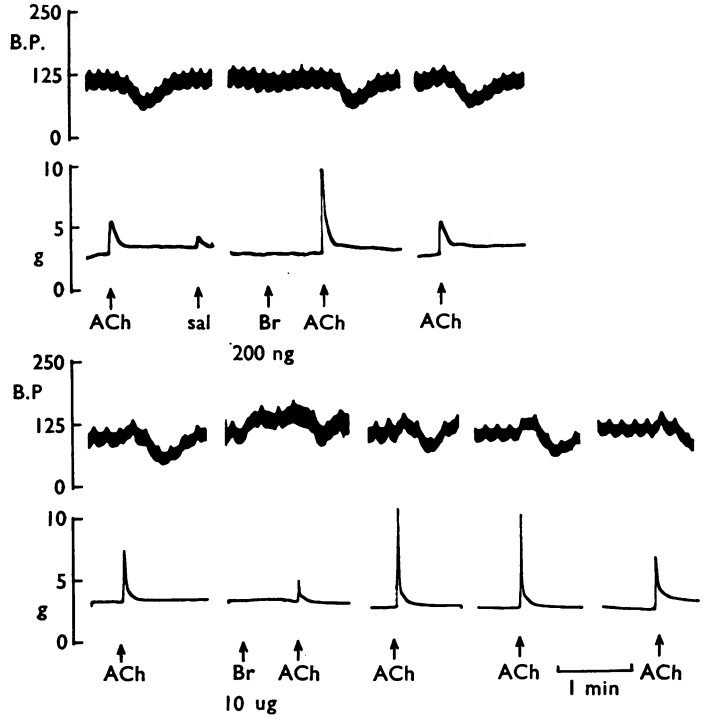

FIG. 7. Cat; chloralose. Upper record: arterial blood pressure. Lower record: tension of denervated tibialis anterior muscle. At (ACh), 4 μg acetylcholine was injected close arterially. The time between acetylcholine injections was 5 min. At (Br) bradykinin 200 ng and 10 μg and at (sal) 0·2 ml of 0·9% w/v NaCl (saline) were injected intra-arterially.

abolished. In the experiment of Fig. 7, 4 μg ACh injected close intra-arterially produced a contracture ; 200 ng bradykinin, though not itself producing a change in tension, increased the response to a subsequent injection of 4 μg ACh. After 5 min, the response to ACh returned to the previous height. 10 μg bradykinin, which was also without effect on tension on its own, seemed at first to have depressed the response to 4 μg ACh. On repeating this dose of ACh every 5 min the response was potentiated and then returned to normal.

Bradykinin in doses of 200–250 ng did not produce any response of the denervated soleus muscle, nor did it modify the effect of subsequent injections of ACh. Effective doses of ACh in these preparations varied from 100 ng to 20 μg.

Discussion

The present experiments show that bradykinin elicits a contractile response in the denervated muscles of the tongue. The similarity with the response produced by ACh suggests that bradykinin may be acting through the release of ACh from the nerve endings ; however, this is not supported by the results with physostigmine, which did not potentiate the contracture produced by bradykinin ; furthermore gallamine had little if any effect on the response to bradykinin, whereas that to ACh was depressed or abolished. The more marked depression of the bradykinin response produced by tubocurarine can be related to the depolarization that this drug produces on denervated muscle (Bowman & Raper, 1964) ; this means that tubocurarine would affect nonspecifically the response to any substance injected shortly afterwards as was seen in the present experiments with sodium nitrite and with isoprenaline.

The effect of tubocurarine on the response to bradykinin can, however, be interpreted in another way, for tubocurarine has a blocking effect on ganglionic transmission. Lewis & Reit (1965) demonstrated that bradykinin stimulates the superior cervical ganglion of the cat, and the tongue of the cat has many parasympathetic ganglia (Fitzgerald & Alexander, 1969) ; therefore, bradykinin could stimulate them and tubocurarine might block this effect. When cocaine was used to block the effect of bradykinin on ganglia (Trendelenburg, 1966) it abolished the contractile response of the tongue, but this result is not conclusive because cocaine also reduced the response to ACh and to chordo-lingual nerve stimulation.

A direct effect of bradykinin on the denervated muscles of the tongue cannot be excluded on the basis of the results obtained with cocaine, which could possibly have some action on denervated muscle, as suggested by the modification that it produced in the contractile response to ACh and chorda stimulation. Moreover, if bradykinin acted only on the ganglia, it would be expected that the effect of the ACh released at the nerve endings would have been blocked by gallamine and tubocurarine, at least to the same degree as was chordo-lingual nerve stimulation. In addition, the contractile effect of bradykinin on the tibialis anterior muscle, where section of the sciatic nerve does not leave any intact nerve endings and is devoid of ganglia, would be in favour of the hypothesis of a direct effect.

The contracture produced by stimulation of the chordo-lingual nerve and the contractile effect of ACh were modified in a similar way by tubocurarine and gallamine. The faster recovery of the contracture produced by nerve stimulation when gallamine was used is in agreement with the usual finding that it is easier to

block the effect of exogenous than of endogenous ACh. With cocaine, it was observed that the decrease in the response to chorda stimulation was more marked than that to ACh. Heidenhain (1883) noted that the stimulation of chorda was accompanied by marked reddening of the tongue; this vasodilatation might be due, at least partly, to bradykinin production, for it has been demonstrated (Hilton & Lewis, 1958) that under these conditions, kinin-forming enzyme is released from the intra-lingual secretory glands; therefore, it is likely that the effects of the stimulation of the chordo-lingual nerve are mediated both by the ACh released and the bradykinin produced.

Adrenaline produces contractile response of denervated muscle (Euler & Gaddum, 1931; Bülbring & Burn, 1936; Ado *et al.*, 1946; Sánchez & Luco, 1956; Bhoola & Schachter, 1961; Bowman & Raper, 1965). It may be argued that the contractile response obtained with bradykinin could be due to catecholamine release; it has been shown by Feldberg & Lewis (1964) that bradykinin releases adrenaline from the adrenal medulla, but neither the small doses injected close intra-arterially into the tongue, nor the time-course of the contracture obtained in the present investigation, would support the theory that adrenaline mediates the responses of the denervated tongue. In addition, Torres, Medina & Amat (1971) have reported that bradykinin produces a contractile response of the denervated tibialis anterior muscle in the adrenalectomized rat.

Denervated muscle responds to a variety of substances: adrenaline, isoprenaline (Bowman & Zaimis, 1961; Bowman & Raper, 1965; Turkanis, 1969), tubocurarine (McIntyre, King & Dunn, 1945; Bowman & Raper, 1964), and histamine (Ado *et al.*, 1946; Loomis & Konker, 1967; Alonso De Florida *et al.*, 1965a, b); these last authors also found responses of the denervated guinea-pig diaphragm to 5-hydroxytryptamine and bradykinin. In our experiments the denervated tongue responded to bradykinin, tubocurarine, gallamine, isoprenaline, sodium nitrite and in some cases to histamine. Normally innervated muscles do not respond to bradykinin or histamine; isoprenaline, although it modifies the maximal twitches of normal muscles, does not produce changes in tension; tubocurarine and gallamine produce their blocking effect on innervated muscle without having any stimulant action; sodium nitrite is known to act only in smooth muscle. It may be suggested that denervation produces qualitative changes in the chemical sensitivity of striated muscle.

We are indebted to Professor S. M. Hilton for his advice and encouragement, and to Dr. G. Vrbová for her valuable suggestions. One of us (B.R.) is grateful to Riker for a fellowship and to the Wellcome Trust for a travel grant. S. H. T. has a fellowship from the "Ministerio de Sanidad y Asistencia Social" through the "Universidad de Oriente", Venezuela.

REFERENCES

Ado, A. D., Ginetsinsky, A. G. & Shamarina, N. M. (1946). The allergic reaction of skeletal muscle. *Fiziol. Zh. SSSR*, **32**, 76–88.

Albuquerque, E. X. & Thesleff, S. (1968). A comparative study of membrane properties of innervated and chronically denervated fast and slow skeletal muscles of the rat. *Acta physiol. scand.*, **73**, 471–480.

Alonso De Florida, F., Del Castillo, J., González, C. C. & Sánchez, V. (1965a). The anaphylactic reaction of denervated muscle in the guinea-pig. *Science, N.Y.*, **147**, 1155–1156.

Alonso De Florida, F., Del Castillo, J., González, C. C. & Sánchez, V. (1965b). On the pharmacological and anaphylactic responsiveness of denervated skeletal muscle of the guinea-pig. *Br. J. Pharmac.*, **25**, 610–620.

Axelsson, J. & Thesleff, S. (1959). A study of supersensitivity in denervated mammalian skeletal muscle. *J. Physiol., Lond.*, **147**, 178–193.

BHOOLA, K. D. & SCHACHTER, M. (1961). Contracture of the denervated rat diaphragm by adrenaline. *J. Physiol., Lond.*, **157**, 20P.

BOWMAN, W. C. & RAPER, C. (1964). Spontaneous fibrillary activity of denervated muscle. *Nature, Lond.*, **201**, 160–162.

BOWMAN, W. C. & RAPER, C. (1965). The effects of sympathomimetic amines on chronically denervated skeletal muscles. *Br. J. Pharmac. Chemother.*, **24**, 98–109.

BOWMAN, W. C. & ZAIMIS, E. (1961). The action of adrenaline, noradrenaline and isoprenaline on the denervated mammalian muscle. *J. Physiol., Lond.*, **158**, 24–25P.

BROWN, G. L. (1937). The actions of acetylcholine on denervated mammalian and frog's muscle. *J. Physiol., Lond.*, **89**, 438–461.

BÜLBRING, E. & BURN, J. H. (1936). The Sherrington phenomenon. *J. Physiol., Lond.*, **86**, 61–76.

DALE, H. H. & GADDUM, H. (1930). Reactions of denervated voluntary muscle, and their bearing on the mode of action of parasympathetic and related nerves. *J. Physiol., Lond.*, **70**, 109–144.

EMMELIN, N. & NORDENFELT, I. (1959). Effects of cross-suture and of injected acetylcholine on the supersensitivity of denervated striated muscle. *Br. J. Pharmac. Chemother.*, **14**, 234–238.

EULER, U. S. & GADDUM, J. H. (1931). Pseudomotor contractures after degeneration of the facial nerve. *J. Physiol., Lond.*, **73**, 54–66.

FELDBERG, W. & LEWIS, G. P. (1964). The action of peptides on the adrenal medulla. Release of adrenaline by bradykinin and angiotensin. *J. Physiol., Lond.*, **171**, 98–108.

FELTZ, A. & MALLART, A. (1971). Ionic permeability changes induced by some cholinergic agonists on normal and denervated frog muscles. *J. Physiol., Lond.*, **218**, 101–116.

FITZGERALD, M. J. T. & ALEXANDER, R. W. (1969). The intramuscular ganglia of the cat's tongue. *J. Anat.*, **105**, 27–46.

FRANK, E., NOTHMAN, M. & HIRSCH-KAUFFMANN, H. (1922). Uber die "Tonische" Kontraktion des querges-treiften Saugetier-muskels nach Ausschaltung des motorischen Nerven. *Pflügers Arch. ges. Physiol.*, **197**, 270–287.

GINETSINSKY, A. G. & SHAMARINA, N. M. (1942). The tonomotor phenomenon in denervated muscle. *Usp. Sovrem. Biol.*, **15**, 283–294.

HEIDENHAIN, R. (1883). Ueber pseudomotorische Nervenwirkungen. *Arch. Anat. Physiol.*, Suppl., 133–177.

HILTON, S. M. & LEWIS, G. P. (1958). Vasodilatation in the tongue and its relationship to plasma kinin formation. *J. Physiol., Lond.*, **144**, 532–540.

LEWIS, G. P. & REIT, E. (1965). The action of angiotensin and bradykinin on the superior cervical ganglion of the cat. *J. Physiol., Lond.*, **179**, 538–553.

LOOMIS, T. A. & KONKER, A. C. (1967). Effects of an organic phosphate on the response of denervated muscle to acetylcholine and some neuromuscular blocking agents. *Arch. int. Pharmacodyn. Ther.*, **165**, 308–318.

MCINTYRE, A. R., KING, R. E. & DUNN, A. L. (1945). Electrical activity of denervated mammalian skeletal muscle as influenced by d-tubocurarine. *J. Neurophysiol.*, **8**, 297–307.

MILEDI, R. (1960). Junctional and extrajunctional acetylcholine receptors in skeletal muscle fibres. *J. Physiol., Lond.*, **151**, 24–30.

PHILIPEAUX, J. M. & VULPIAN, A. (1863). Note sur une modification physiologique que se produit dans le nerf lingual par suite de l'abolition temporaire de la motricité dans le nerf hypoglosse du même côte. *C.r. hebd. Séanc. Acad. Sci., Paris*, **lvi**, 1009.

ROSENBLUETH, A. & LUCO, J. V. (1937). A study of denervated mammalian skeletal muscle. *Am. J. Physiol.*, **120**, 781–797.

SÁNCHEZ, P. & LUCO, J. V. (1956). Study of curare-acetylcholine antagonism in normal and denervated auricular muscles. *Acta neurol. latinoam.*, **3**, 189–193.

TORRES, S. H., MEDINA, A. N. & AMAT, J. (1971). Response of denervated tibialis anterior muscle of the rat to smooth muscle stimulating substances. In: *Proceedings of the International Union of Physiological Sciences*, **9**, 1960.

TRENDELENBURG, U. (1966). Observations on the ganglion-stimulating action of angiotensin and bradykinin. *J. Pharmac. exp. Ther.*, **154**, 418–425.

TURKANIS, S. A. (1969). Some properties of the denervated anterior gracilis muscle of the rat. *Br. J. Pharmac.*, **37**, 414–424.

(*Received May* 4, 1972)