and sharper with the IR light (figure 1c). The relative improvement with IR illumination was more pronounced with thicker muscles.

IR diffraction spectra were often obtained from thicker muscles which showed no visible diffraction spectra. Figure 2a illustrates the IR diffraction spectra from such a case, a rabbit papillary muscle whose thickness (750 μm) was 3 times that of specimens giving comparable spectral clarity with helium neon laser illumination[7]. The use of IR light thereby permits direct comparison of the effects of sarcomere motion upon contractile behavior in mammalian heart muscles which have different intracellular pathways of contractile activation and control. For example, the maximum sarcomere shortening in another rabbit papillary muscle (2.30–1.65 μm, figure 2b) is comparable to that seen in rats[11], although the onset after stimulation is appreciably delayed (i.e., 40 msec vs 15 msec). No deleterious effect of IR illumination upon contractile force was noticed over the 3 h of observation. Improved spectral clarity with IR illumination suggests that the dispersion of first order light is affected more by low angle scattering than by the actual sarcomere length distribution within the muscle. When a uniform grating was illuminated by light passing first through the muscle, the dispersion of first order spectra was equivalent to the intensity distribution of figure 1c. Direct illumination of the grating produced equivalent, narrow dispersion for both light sources. Thus, the zero order light collimation is compromised at each diffracting layer of the muscle tissue, and the actual distribution of sarcomere lengths must be narrower than the light intensity dispersion shown in figure 1c. This speculation is supported by direct measurements by others which show little sarcomere length dispersion in living papillary muscles at rest[12].

Despite uncertainties about inference of sarcomere length distribution by diffractometry, it is often possible to detect discrete sarcomere lengths in these preparations. Since the light emitted by the gallium arsenide laser diode has a finite spectral bandwidth (3.5 nm), the IR diffraction patterns are not as speckled[13] as those obtained from the helium neon laser (refer to lack of small intensity fluctuations on figure 1a as compared to 1b). In the absence of speckle noise it is often possible to resolve small peaks which occur only on that part of the diffraction pattern where a component due to sarcomere diffraction would be predicted (figure 3). The position of these peaks is not stationary and may be related to the presence of small, nonpropagated foci of spontaneous contractile activity seen late during the interval between contractions in intact[7,14] and skinned[15] muscle preparations.

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GABA in the caudate nucleus: A possible synaptic transmitter of interneurons[1]

R. Spehlmann, K. Norcross and E. J. Grimmer[2]

VA Lakeside Hospital and Departments of Neurology and Pharmacology, Northwestern University Medical School, Chicago (Illinois 60611, USA), 3 November 1976

Summary. Microiontophoretic application of GABA and its antagonist, picrotoxin, altered focal potentials evoked in the caudate nucleus by stimulation near the recording site to a much greater extent than potentials elicited by stimulation of afferent pathways, suggesting that GABA is a transmitter of interneurons in this nucleus.

The possibility that gamma-aminobutyric acid (GABA) acts as a neurotransmitter in the caudate nucleus (CN) is suggested by the fairly high concentration of GABA and its synthetizing enzyme, glutamic acid decarboxylase (GAD) in this nucleus[3–5]. GABA and GAD are reduced in the CN in Huntington's chorea[6,7], which is characterized by a loss of interneurons in the CN, and in Parkinson's disease[8,9], suggesting that striatal GABA plays a role in the pathophysiology of human movement disorders. When applied microiontophoretically to caudate neurons, GABA reduces their firing rate[10,11] and mimics the effect of synaptically induced inhibition[12]. Possibly, some GABA-containing terminals in the CN are recurrent collateral fibers from striato-nigral connections; these connections were first considered GABAergic because the GABA antagonist, picrotoxin, blocks focal evoked potentials and the depression of neuronal firing produced in the substantia nigra by stimulation of the CN[13,14]. However, it is unlikely that recurrent collaterals account for the high concentration and widespread effects of GABA in the CN, since less than 5% of the caudate neurons give rise to efferent fibers[15]. Moreover, because lesions of major connections to and from the CN do not reduce caudate GABA or GAD levels, it has been sug-

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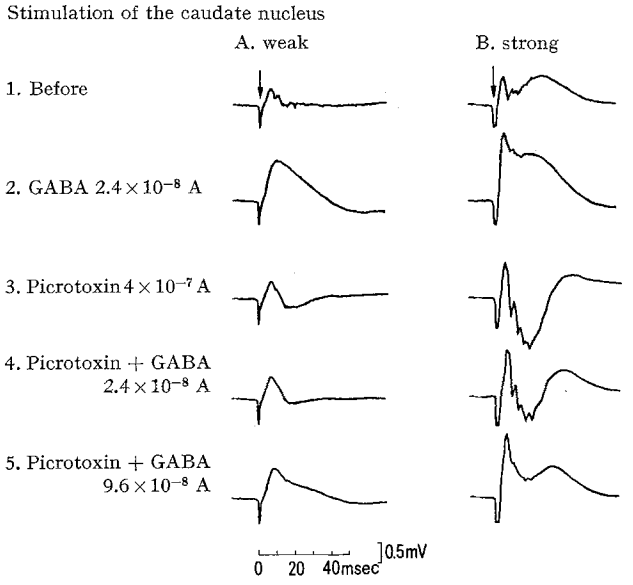

Fig. 1. The effects of GABA and picrotoxin on focal potentials evoked in the caudate nucleus by weak and strong stimulation near the recording site.

gested that most of the GABA-containing endings in the CN arise from interneurons[16]. We therefore compared the effects of GABA and picrotoxin on responses of the CN to stimulation within this nucleus and to stimulation of its major afferent connections and offer electrophysiological evidence supporting the role of GABA as a transmitter of caudate interneurons.

Cats were prepared under general anesthesia and the spinal cord was transected at C-1. Thereafter, animals were maintained on local anesthesia to wound margins and pressure points in order to avoid the effects of general anesthesia on neuronal responses. Blood pressure, heart rate, temperature and CO_2 level were monitored. 8-barreled micropipettes with total tip diameters of 3–6 micra were used as in previous studies[17]. The central recording barrel contained K citrate (1.5 M, pH 7), the surrounding drug barrels, GABA (4 M, pH 4), picrotoxin (5 mM in 165 mM NaCl, pH 5.2), Na L-glutamate (2 M, pH 8) and NaCl (3 M, pH 7). Drug ions were liberated from the micropipette by currents of appropriate polarity and intensities up to 2×10^{-7} A. Between applications drug ions were retained with weak currents of opposite polarity. All currents were returned through the NaCl barrel to avoid possible electrical effects of the iontophoretic currents. The micropipette was inserted stereotaxically into the head of the CN (A 14.5–18.5; L 2–5; D 5–1) and advanced parallel to, and 2–5 mm from, a stimulus electrode having 4 electrical contacts at different levels in the nucleus. Bipolar stimulus electrodes were placed into nigrostriatal connections (A 7.5; L 5; D – 3.5) and the nucleus ventralis anterior thalami (A 11; L 5; D 3.5); a platinum ball electrode was placed on the pericruciate cortex.

Stimulation of the CN commonly produced a positive focal evoked potential at the micropipette tip, a few mm from the stimulus electrode. Microiontophoretic application of GABA at the recording site consistently affected these potentials when the micropipette tip was located near the soma of a caudate neuron, as demonstrated by recording spontaneous or glutamate-induced firing of extracellular action potentials.

Weak stimulation of the CN (figure 1A) produced a potential having an early peak at 5–10 msec. GABA augmented this potential, especially its late part. While picrotoxin alone had little effect on the evoked potential, it antagonized the action of GABA. This antagonism could be overcome by increasing doses of GABA. Strong stimulation of the CN (figures 1B and 2A) added late components to the early peak of the evoked potential; these late components were also greatly enhanced by GABA. While picrotoxin alone had little effect on the early peak, it abolished late components at 10–25 msec (figure 2A) and, at high doses (figure 1B), inverted them. GABA antagonized these effects.

Stimulation of the major afferent pathways also elicited positive focal potentials in the CN (figure 2) with slightly longer latencies than the responses to stimulation of the CN. GABA and picrotoxin altered the components at 10–25 msec of these potentials in the same fashion, but to a lesser degree, than they altered responses to caudate stimulation recorded in the same electrode position.

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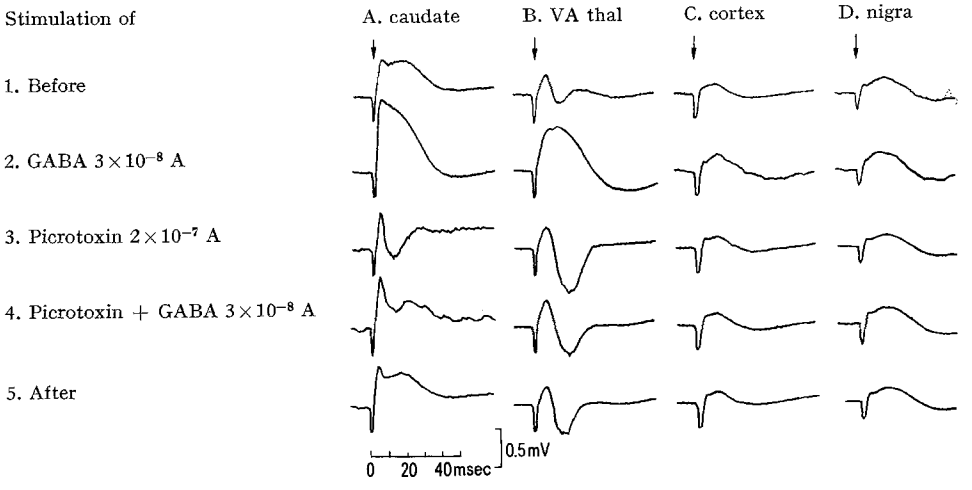

Fig. 2. The effects of GABA and picrotoxin on focal potentials evoked in the caudate nucleus by stimulation of the caudate nucleus, nucleus ventralis anterior thalami, pericruciate cortex and substantia nigra.

Responses to thalamic stimuli were more susceptible than those to stimulation of the pericruciate cortex, and responses to nigral stimulation were usually least affected. The observation that responses to stimulation of the CN are more sensitive to GABA and picrotoxin than are responses to stimulation of major afferent pathways suggests that these agents act preferentially on the output of caudate interneurons and not on the input to these neurons from major afferents. This action was clear in spite of factors which could bias results in the opposite direction, namely: a) while direct stimulation of the CN principally excites interneurons, it also excites some afferent fibers traversing the CN, and b) stimulation of afferent pathways secondarily activates some interneurons and may account for the weaker effects of GABA and picrotoxin seen in potentials evoked by stimulation of the afferent pathways.

The effects of picrotoxin further support the concept that intrinsic GABA is involved in the production of the response to stimulation of caudate interneurons. Picrotoxin antagonized the effect of extrinsic GABA on the evoked potential, as would be expected of an antagonist of any agent affecting the evoked potential. More importantly, picrotoxin alone antagonized and inverted those components of the evoked potential which were facilitated by extrinsic GABA; the inversion is probably due to components of opposite polarity normally masked by the GABA-sensitive components. The block of GABA-sensitive components by picrotoxin makes it likely that picrotoxin interacts with an intrinsic GABA receptor which is responsible for the production of the late components. Because these components were elicited only by strong stimuli and had a longer latency than the early peak produced by weak stimuli, they are presumed to be due to the excitation either of interneurons of relatively high threshold and slow conduction velocity or of polysynaptic interneuronal connections. While GABA may thus be the synaptic transmitter of interneurons of this description, another transmitter is probably liberated by a set of interneurons with a low threshold producing the early peak in the evoked potential. The GABA receptor is probably at or near the soma of interneurons, because picrotoxin altered evoked potentials only when applied at a site where neuronal action potentials could also be recorded. This is consistent with electronmicroscopic evidence showing that afferent fibers terminate on dendrites and somata of interneurons while terminals of interneurons have synapses on the initial segment as well[18].

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The effect of vasoactive agents on nutritive collateral circulation in rat muscles

L. Takács, B. Kenéz, L. Debreczeni and Karola Albert

2nd Department of Medicine, Semmelweis University Medical School, Szentkirályi-u. 46, H-1088 Budapest (Hungary), 16 July 1976

Summary. The relationship between the collateral and control nutritive blood flow (^{86}Rb) of the triceps muscle was not influenced by vasoactive agents (phenylephrine, angiotensin II, vasopressin) in the rat. Isolevine caused vasodilation only in the control muscle.

Immediately following ligation of an artery, the oxygen and metabolic supply of the area fed by that artery is determined by vessels called primary nutritive collaterals. Both from physiological and clinical points of view, it is important to know the sensitivity and reactivity of the nutritive collaterals. In our experiments, the effect of vasoactive agents on the accumulation of ^{86}Rb by m. triceps surae, following closure of the femoral artery, has been studied.

Methods. Male rats after 12–16 h of fasting were anesthetized with 50 mg/kg b.wt pentobarbital i.p. Arterial blood pressure was continuously recorded from the carotid artery with a mercury manometer. Cardiac output was determined with the Evans blue dilution method, and the m. triceps surae fraction of the cardiac output was measured using the isotope fractionation method of Sapirstein[1]. Circulatory resistance was calculated. Nutritive collateral resistance refers to resistance of the whole area accumulating Rb and supplied by the collaterals. Since we do not know the pressure drop inside individual vessel segments, partial resistances cannot be calculated from our data. Details of these methods are found in our previous papers[2,3].

The left femoral artery was ligated under the inguinal ligament. In previous experiments[2,3], it was found that the resistance of the collaterals of the m. triceps surae does not change during the first 10–120 min following ligation. Therefore, we started the infusion of drugs into the jugular vein 10 min after ligation. The infusion lasted 5 min, at a rate of 0.02 ml/min, after which the cardiac output was determined by sampling blood from the carotid artery; then about 10 μCi of ^{86}Rb was injected into the jugular vein. At 60–80 sec after Rb injection, the animals were killed by i.v. administration of KCl solution. The cumulative doses of the vasoactive agents for 100 g b.wt were as follows: vasopressin: 16 and 32 mU; angiotensin II: 0.10 and 0.20 μg; phenylephrine: 9.2 and 19.4 μg; isolevine: 0.11 and 0.19 μg. Cardiac output and TPR were calculated for 100 g b.wt, blood flow and resistance of the triceps muscle for 100 g muscle weight. Changes in the experimental animals were compared to those in the non-treated control group. Data are shown in the table with common standard deviations.

Results and comments. As illustrated in the table, the nutritive blood flow to the left triceps muscle decreased to 55% and its resistance increased to 206% of the contralateral side following ligation of the left femoral artery. Both concentrations of vasopressin, angiotensin II and phenylephrine increased the blood pressure and increased the resistance of the triceps muscle on both the ligated

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