ever, in rats, goats and sheep, complete transection of the pituitary stalk induces extensive bilateral necrosis of the pars distalis, whereas partial transection of the stalk causes only unilateral necrosis on the corresponding side of the pars distalis[10]. This possibly suggests that certain groups of portal vessels supply restricted areas in the pars distalis. It is likely that such a situation may also prevail in birds[11].

Zusammenfassung. Die ermittelte Gefässversorgung der Vogelhypophyse beweist die Existenz eines doppelten Portalsystems der Pars distalis, womit auf zwei hypothalamo-hypophysäre Mechanismen hingewiesen wird.

RAM MURAT SINGH and C. J. DOMINIC

Department of Zoology, Banaras Hindu University, Varanasi 5 (India), 22 January 1970.

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Choline Acetyltransferase and Acetylcholinesterase in Myo-Tendinous and Neuro-Muscular Junctions of Mouse Skeletal Muscle

Acetylcholine (ACh), choline acetyltransferase (ChAc) and acetylcholinesterase (AChE), are highly concentrated at the neuro-muscular junctions of skeletal muscle fibres[1,2]. It has been shown further that AChE is highly concentrated also at the junctions between skeletal muscle fibre and tendon[3–8], and that in some muscles the membrane is more sensitive to locally applied ACh in this region than elsewhere (except in the neural zone)[9–11]. It is unknown, however, whether ACh has a function at the myo-tendinous junctions. As preformed ACh probably cannot diffuse to the myo-tendinous region from other places, we wanted to determine the activity of ChAc, the enzyme synthesizing ACh, in this region.

The external oblique abdominal muscle of albino mice was used. In this thin segmental muscle, both neuromuscular and myo-tendinous junctions are located in discrete, narrow bands. The muscular abdominal wall was pinned on carboard coated with aluminium foil and frozen by floating on liquid N_2. Such treatment makes neuro-muscular and myo-tendinous junctions easily visible[12]. Stable and easily handled preparations were obtained on freeze-drying. Approximately 0.3×3 mm samples of the external oblique muscle were dissected under a stereo microscope (Figure 1) by means of razor blades and pointed tweezers. Adhering pieces of underlying muscles were easily recognized and removed from the samples. The dissection was occasionally controlled by staining the remaining tissue for AChE with a modified Koelle-Friedenwald technique[13]. The samples were weighed (10–60 µg) on a Cahn Gram electrobalance. Samples containing neuro-muscular junctions, myo-tendinous junctions and non-junction muscle were assayed for AChE and ChAc by radiochemical methods based on the hydrolysis of [1-^{14}C]acetylcholine chloride (The Radiochemical Centre, Amersham, England) and on the production of ACh from [1-^{14}C]acetyl-CoA (New England Nuclear Corp., Boston, Mass.)[14]. For AChE the samples were incubated for 30 min at 25 °C in 25 µl of incubation mixture, for ChAc the figures were 60 min, 37 °C and 25 µl. Triton X-100 was added at 0.5% (v/v) final concentration to release all enzyme activity. Other details were as earlier described[14].

The AChE activity was 4–9 times higher in the neuromuscular and myo-tendinous preparations than in those from non-junction muscle (Figure 2). For ChAc the ratios of the mean activities of samples from neuro-muscular junctions, non-junction muscle and myo-tendinous junctions were 100:6:4 (Figure 2). The same activity ratios (100:7:5) were obtained in additional experiments with a method using [1-^{14}C]acetate and an enzyme system to generate [1-^{14}C]acetyl-CoA in situ[14], although in these experiments the absolute activities were somewhat higher. With either method the activity of the myo-tendinous

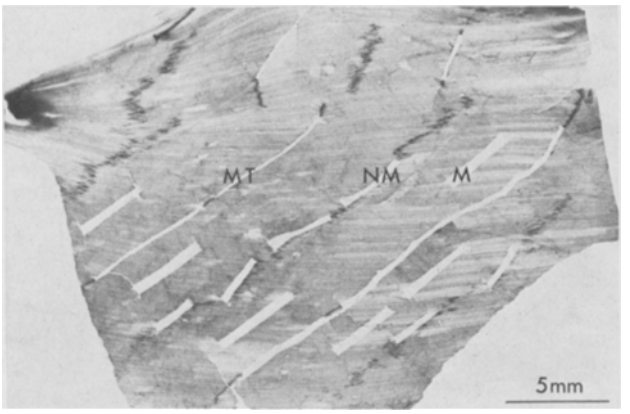

Fig. 1. Photomicrograph of the freeze-dried external oblique abdominal muscle after dissection of samples containing neuro-muscular junctions (NM), non-junction muscle (M) and myo-tendinous junctions (MT). Note that the MT could be dissected thinner than the NM and therefore were less diluted with muscle. Note also that the MT contained the junctions from 2 adjacent segments.

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samples was just within the limit of detection. Part of the low ChAc activity in non-junction and myo-tendinous samples may be due to intramuscular nerve branches.

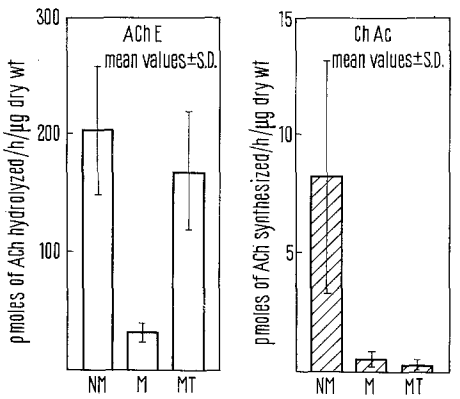

Fig. 2. Activities of AChE and ChAc in the dissected samples NM, M and MT (symbols as in Figure 1). From 3 animals 15 samples of the various kinds were assayed for each enzyme.

The above results establish that there is a close correspondence between the ChAc and AChE activities in neuro-muscular junctions, whereas in the myo-tendinous junctions a high AChE activity is contrasted to the almost total lack of ChAc. From these observations we conclude that ACh is not synthesized near the myo-tendinous junctions and that the AChE present there does not normally hydrolyse ACh.

Zusammenfassung. Topographischer Nachweis hoher Acetylcholinesterase-Aktivität an der Übergangsstelle Muskel/Sehne und, im Unterschied zur neuromuskulären Verbindung, an dieser Stelle kaum messbare Aktivität der Cholinacetyltransferase, was ohne Beziehung zur cholinergen Erregungsübertragung steht.

P. R. Flood, F. Fonnum
and J. Storm-Mathisen

Norwegian Defence Research Establishment, Division for Toxicology, N-2007 Kjeller (Norway), 15 February 1970.

Evidence for a Spinal Sympathetic Regulation of Cardiovascular Functions[1]

The reflex control of cardiovascular functions is commonly thought to be exclusively integrated at supraspinal levels. Spinal sympathetic reflexes, though known to exist[2], are not considered to be activated by haemodynamic events. Sympathetic rami, however, represent a simple pathway through which afferent information from heart and blood vessels may reach the cord and reflex signals may be returned to the cardiovascular system without involvement of the brain stem. Reflexes of this type might subserve local segmental regulation of cardiovascular functions much more aptly than the classical medullary reflexes involved in the overall control of systemic circulation.

These reflexes could be conveniently studied by recording the electrical activity of the sympathetic rami. Indeed, we have recently shown[3] that transient coronary occlusion in cats can modify the activity of a sympathetic preganglionic outflow to the heart (i.e. the third left thoracic ramus communicans, T3, which is known to contribute significantly to the efferent innervation of the heart[4] and particularly to myocardial contractility[5]). These effects were reflex in nature and consisted most often in increases in sympathetic discharges. They were found to be independent of vagal fibres and present in intact, decerebrate and spinal preparations. This was the first demonstration of a cardio-cardiac spinal sympathetic reflex. In order to investigate whether cardiac sympathetic spinal reflexes can also be elicited by a more normal haemodynamic event, and therefore participate in the physiological rather than pathological regulation of circulation, we have studied the effects on the same sympathetic outflow of changes in arterial blood pressure. As will be described, increases in arterial blood pressure can reflexly modify the activity of T3 fibres in spinal vagotomized preparations.

Experiments were performed on 24 cats, anaesthetized by i.p. injection of pentobarbital sodium (35 mg/kg). Preparations were paralyzed with gallamine triethiodide and artificially ventilated. In all cats the spinal cord was sectioned at C1 and both vagi cut. The left stellate ganglion was exposed retro-pleurally and T3 was dissected under a microscope into tiny filaments until a single or a few active fibres could be isolated. Electrical activity of the sympathetic fibres was suitably amplified and recorded on film from CRO screen, simultaneously with carotid arterial pressure. Other recording details have been described[3]. The rises in arterial pressure, obtained either by stenosing or occluding the thoracic aorta at various sites (by pulling a ligature placed around it) or by i.v. injection of pressor drugs (noradrenaline and angiotensin) were usually tested several times for each fibre and for each multifibre preparation.

We studied the activity of 54 single preganglionic sympathetic fibres and 34 multifibre preparations (Table). This Table shows that single fibres could respond to rises in arterial pressure either by decreasing or increasing their firing rate, with some predominance for the first type of response. A reduction in firing rate was obtained far more frequently from multifibre preparations. For each single fibre and multifibre preparation the type of response was consistent through several and different trials.

The Figure shows an example of reduction in firing rate of a single fibre following occlusion of the thoracic aorta. This type of response was found to be graded according to the amplitude of the blood pressure rise, however obtained (by constricting the aorta or by injecting pressor drugs). Since this response might have

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