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Synaptic Transmitters and Receptors: Selected Aspects of Recent Research in Czechoslovakia

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Several laboratories have been involved in the research of synaptic transmitters and receptors during the post-World War II period in Czechoslovakia. The main contributions concerned the role of acetylcholine in the heart, motor nerves, skeletal muscles and the intestine, the synthesis of acetylcholine in the nerve terminals and its release, the role of catecholamines and the sympathoadrenal system in stress, the function of adenylate cyclase and cyclic AMP, and the function of muscarinic acetylcholine receptors and N-methyl-D-aspartate (NMDA) receptors. It would be difficult to provide a complete review of the work performed; some of its most interesting aspects are only mentioned in the following text.

Acetylcholine in the heart

It was discovered by Vlk (1958a) that there is considerably more acetylcholine in the heart atria than in the ventricles. Later, a gradient in the distribution of acetylcholine in the heart atria was established (Vlk 1958b, Vlk and Tuček 1961, Vlk *et al.* 1961), with acetylcholine concentration decreasing in the order: sino-atrial node > right atrium > left atrium. A similar distribution was also discovered for choline acetyltransferase, the enzyme responsible for the synthesis of acetylcholine (Tuček 1964, Slavíková and Tuček 1982a, 1985).

Biochemical data were obtained, providing a partial explanation why the tonic influence of vagal nerves (Lhoták 1911, Vlk 1966, Slavíková and Tuček 1982b) and the effect of vagal stimulation upon the heart (Babák and Bouček 1907, Vlk and Vincenzi 1977, Vlk 1979) increase during ontogenetic development. The content of acetylcholine in the heart (Vlk 1958a, Vlk and Tuček 1962, Kuntscherová and Vlk 1979) and the activity of choline acetyltransferase (Tuček 1965, Slavíková and Tuček 1982b) undergo a marked increase during the postnatal period, while the density of muscarinic receptors slightly decreases (Nedoma *et al.* 1986).

Acetylcholine in motor nerves and skeletal muscles

Much of the work concerning the release of acetylcholine from motor nerve terminals and the function of nicotinic acetylcholine receptors in skeletal muscles is being mentioned elsewhere in this issue (see the article by P. Hník). Radioenzymatic measurements of the acetylcholine content in skeletal muscles provided a confirmation of earlier findings obtained with bioassays, indicating that

acetylcholine is present in skeletal muscles not only in the motor nerve terminals, but also in the skeletal muscle fibres (Doležal and Tuček 1983). The enzyme responsible for the synthesis of acetylcholine in muscle fibres is not choline acetyltransferase but rather carnitine acetyltransferase (Tuček 1982). The role of the acetylcholine which is being produced in the muscle fibres has not been clarified. The total (neural and muscular) capacity for the synthesis of acetylcholine in skeletal muscles varies considerably during postnatal development and aging (Tuček 1972, Tuček and Gutmann 1973) as well as after denervation (Tuček 1973, 1982). It is affected by testosterone and castration in the androgen-sensitive levator ani muscle in rats (Gutmann *et al.* 1969, Tuček *et al.* 1976a,b). The transection of frog (but not mammalian) nerves triggers the synthesis of choline acetyltransferase in the cells of Schwann of the degenerating nerve trunks (Tuček *et al.* 1978a). Choline acetyltransferase in the nerve trunks is affected by ischaemia (Tuček *et al.* 1978b, Malátová *et al.* 1989).

The spontaneous non-quantal release of acetylcholine from motor nerve terminals, first discovered in frog muscles (Katz and Miledi 1977) was subsequently found to proceed also in mammalian muscles (Vyskočil and Illés 1977, 1978, Vizi and Vyskočil 1979). The spontaneous non-quantal release is mediated by carriers, which are inhibited by botulinum toxin (Doležal *et al.* 1983) and by 2-(4-phenylpiperidino)-cyclohexanol (AH 5183, vesamicol; Edwards *et al.* 1985). The sensitivity of the spontaneous non-quantal release to vesamicol known to block the transport of acetylcholine into synaptic vesicles (see Říčný and Collier 1986), suggests that the carriers responsible for the accumulation of acetylcholine in synaptic vesicles may also be responsible for the non-quantal transport of acetylcholine from the nerve terminals if they become incorporated into the presynaptic plasma membranes during vesicular exocytosis. Spontaneous calcium-independent release of acetylcholine was also discovered in synaptosomes prepared from the electric organ of Torpedo; it can be inhibited by muscarinic receptors (Doležal *et al.* 1988).

Acetylcholine in the intestine

Investigations of the release of acetylcholine from the cholinergic neurones in the myenteric plexus led to the finding that the release can be enhanced by prostaglandin E_2 (Kadlec *et al.* 1974, 1978) and to the discovery of post-tetanic potentiation on muscarinic synapses (Kadlec *et al.* 1979, 1982); substance P or a similar compound plays a role in the mechanism of potentiation (Kadlec *et al.* 1984a, Ševčík *et al.* 1990). Investigations on the topography of acetylcholine release from the myenteric plexus (Kadlec *et al.* 1984b, 1985, 1986, 1987a) led to the conclusion that the release depends on the propagation of action potentials along the varicose fibres of the plexus; action potentials may fail to reach distal varicosities during slow stimulation, but their spreading may be improved by cholinomimetics augmenting potassium conductance (Kadlec *et al.* 1990b, 1991) or by high frequency stimulation in the presence of drugs promoting calcium conductance (Kadlec *et al.* 1987b, 1990a).

The synthesis of acetylcholine in neurones

In experiments with subcellular fractionation of brain homogenates, the highest activity of choline acetyltransferase was found in the fraction of the nerve endings (Tuček 1967a,b), in accordance with data indicating that acetylcholine is mainly produced in the nerve terminals. The enzyme, however, is synthesized in neuronal perikarya and is transferred to the nerve terminals by means of slow unidirectional axonal transport (Tuček 1974, 1975). The enzyme is non-specifically activated by common cations (Morris and Tuček 1966).

The question of the origin of the acetyl groups in the molecule of acetylcholine has been given much attention. While there is no doubt that the immediate precursor of these acetyl groups is acetylcoenzyme A (Tuček 1978), the provenience of the acetyl groups in the acetylcoenzyme A which is used for the synthesis of acetylcholine is less clear. In experiments with intracisternal injections of various labelled precursors of acetyl groups to rats *in vivo*, labelled atoms from glucose, pyruvate and lactate were found to be most efficiently incorporated into brain acetylcholine (Tuček and Cheng 1970, 1974). In the electric organ of Torpedo, the most efficient precursor proved to be acetate (Israel and Tuček 1974). Glucose is converted to acetylcoenzyme A *via* pyruvate, which is known to undergo oxidative decarboxylation in the mitochondrial matrix. Three ways are available in which the acetyl groups from the intramitochondrial acetylcoenzyme A may be transferred to the pool of extramitochondrial (cytosolic) acetylcoenzyme A: (a) using citrate as the carrier (Tuček *et al.* 1981, Říčný and Tuček 1982); (b) using acetylcarnitine as the carrier (Doležal and Tuček 1981); (c) utilizing direct passage of acetylcoenzyme A through mitochondrial membranes *via* calcium-dependent hydrophilic channels (Tuček 1967, Říčný and Tuček 1983).

The rate of the synthesis of acetylcholine in brain slices has been found to depend on the concentration of acetylcoenzyme A in the slices (Říčný and Tuček 1980, 1981). The availability of acetylcoenzyme A is likely to be one of the rate-limiting factors in the control of acetylcholine synthesis in the brain *in vivo* (Doležal and Tuček 1982). Other factors involved in the control of the rate of acetylcholine synthesis in cholinergic neurones are the availability of choline acetyltransferase (Tuček 1984, 1985, 1988). In the context of the analysis of the control of acetylcholine synthesis, the finding by Kuntscherová (1972) that the levels of acetylcholine in the brain, heart and intestine may be augmented by the administration of a large dose of choline, was of outstanding interest.

Catecholamines and catecholaminergic systems in stress

Important data have been obtained in studies of peripheral sympathoadrenal and cerebral catecholaminergic systems in animals exposed to different stressors. Changes in the activity of these systems during adaptation to repeated stress have been elucidated as manifested by changes in tissue concentrations of catecholamines, in their secretion, excretion, synthesis and degradation, and in the activity of enzymes involved in these processes (Kvetňanský 1980, Kvetňanský *et al.* 1984). New findings (many of which were obtained in collaboration between the group of R. Kvetňanský in Bratislava and the Laboratory of Clinical Science, NIH, Bethesda, U.S.A.) substantially contributed to the understanding of the mechanisms

regulating the activities of enzymes synthesizing and degrading catecholamines and controlling their secretion, as well as to the understanding of the sources of circulating catecholamines and of the role of brain catecholamines in neuroendocrine processes during acute and repeated stress (Kvetňanský 1973, Kvetňanský *et al.* 1977, 1979a, 1989).

An elevation of the activity of dopamine-β-hydroxylase in the blood plasma was found in animals during stress, in addition to the reduction in tissue concentration of catecholamines and to the elevation of their secretion and excretion. This finding (Weinshilboum *et al.* 1981) was the first published report that dopamine-β-hydroxylase may serve as an indicator of the sympatho-adrenal system activity under stress. It was found that plasma catecholamines are highly elevated in rats killed by decapitation. Therefore, a stress-free procedure for the collection of blood *via* a permanent catheter was devised; this permitted to demonstrate, for the first time, changes in plasma levels of catecholamines during habituation to psychoemotional stimuli (Kvetňanský *et al.* 1978a, Dobrakovová *et al.* 1989).

Investigation of the sources of circulating catecholamines released under stress showed that the elevation of plasma epinephrine is almost exclusively due to its release from the adrenal medulla; plasma norepinephrine is mainly released from the peripheral sympathetic nerve endings, and plasma dopamine originates from both sources (Kvetňanský *et al.* 1979a). In repeatedly stressed rats, an elev D store of catecholamines was found both in adrenal medulla and in sympathetically innervated organs, together with elevated plasma and urine levels of catecholamines (Kvetňanský and Mikulaj 1970, Kvetňanský *et al.* 1984). These changes are the consequence of increased synthesis and most probably also of reduced degradation of catecholamines induced by repeated stress.

The elevated synthesis of catecholamines in repeatedly stressed animals was confirmed by *in vivo* administration of their radioactive precursors (Kvetňanský *et al.* 1971a) and by *in vitro* analyses which showed elevations of activities of synthesizing enzymes (Kvetňanský *et al.* 1970, 1971b). The highly elevated activity of tyrosine hydroxylase, the rate-limiting enzyme in the biosynthesis of catecholamines, in the adrenals of repeatedly stressed rats represents the first published report concerning the activation of this enzyme by physiological stimuli (Kvetňanský *et al.* 1970). Studies of mechanisms involved in the regulation of the elevated biosynthesis of catecholamines showed that the synthesis is regulated by both neural and endocrine factors (Kvetňanský 1973). The notion that cyclic AMP is involved in the regulation of tyrosine hydroxylase activity (Kvetňanský *et al.* 1971c) has become generally accepted. It was also shown that the adrenocortical hormones are involved in the regulation of synthesis, secretion and excretion of catecholamines (Kvetňanský 1973, Kvetňanský *et al.* 1979b).

In studies of the adrenergic system in the brain, particularly in the hypothalamus, it was found that elevated synthesis in combination with reduced degradation are the basic mechanisms responsible for the rise of the concentration of catecholamines in the brain of repeatedly stressed rats; alterations in the concentrations of norepinephrine, dopamine and epinephrine in isolated hypothalamic nuclei of rats were demonstrated (Kvetňanský *et al.* 1977, 1978b, 1983).

In studies performed on cosmonauts and rats during spaceflights, it was found that sympathoadrenal activity is not significantly changed and that long-term

weightlessness does not represent an intensive stressogenic stimulus (Kvetňanský *et al.* 1981). During the long-term stay in weightlessness, however, the sympathoadrenal system becomes more sensitive to stressors. This is manifested by its increased activity after the landing on Earth (Kvetňanský *et al.* 1981, 1988).

Cyclic AMP

Considerable effort has been spent on investigations of the control of cyclic AMP formation and of its physiological role. Persistent activation of adenylate cyclase by the toxin of Vibrio cholerae has been discovered by Hynie and Sharp (Sharp and Hynie, 1971, Hynie and Sharp 1972) and the importance of cyclic AMP in the control of lipolysis and of fatty acid mobilization has been demonstrated (Hynie *et al.* 1966, Brodie *et al.* 1966, 1969, Krishna *et al.* 1968). New data on the hormonal control of enzymes involved in transmembrane signal transduction in the hypophysis (Klenerová and Hynie 1974) have been obtained and the effects of the derivatives of adenosine (including adenosine nucleolipids) on these enzymes have been discovered (Hynie and Smrt 1975, 1978, Smrt and Hynie 1978). The role of cyclic AMP in the control of intraocular pressure and the effects of drugs and peptides on the activity of adenylate cyclase in ciliary processes were investigated by Čepelík and Hynie (1990a,b). Factors affecting membrane fluidity were found to have an effect on the activity of adenylate cyclase (Hynie 1984, Hynie *et al.* 1985).

Both cyclic AMP and cyclic GMP levels were found to increase at the onset of pharmacologically induced clonic seizures and during subsequent tonic convulsions, returning back to control values after the end of seizures (Folbergrová 1975b, 1977, 1980). The elevation of cyclic AMP is mainly due to the interaction of catecholamines with adenylate cyclase through β-adrenergic receptors (Folbergrová 1977, 1981). Cyclic AMP was shown to play an important role in triggering glycogenolysis in the brain during seizures. A close correlation was found between the levels of cyclic AMP and the activation of glycogen phosphorylase in the brain during seizures, and also when the seizures were prevented by pentobarbital (Folbergrová 1975a,b,c, 1977). Some features of the data obtained indicate, however, that a mechanism of phosphorylase activation independent of cyclic AMP is also operating in the brain in which Ca^{2+} ions are likely to play a role (Folbergrová 1981).

Muscarinic acetylcholine receptors

The negative chronotropic effect of the activation of muscarinic receptors in the heart disappeared entirely in animals that had been injected with pertussis toxin, which is known to block the interaction between receptors and certain G proteins (Tuček *et al.* 1987); pertussis toxin apparently blocked the G protein-mediated interaction between muscarinic receptors and K^+ channels in the heart. The G protein is also likely to act as an intermediate in the chain of events which are responsible for the autoinhibition of acetylcholine release from cholinergic nerve terminals mediated by presynaptic muscarinic autoreceptors. In experiments on the myenteric plexus, pretreatment with pertussis toxin entirely abolished the inhibitory effect of muscarinic activation on the release of acetylcholine evoked by depolarization of the plexus (Doležal *et al.* 1989). Presynaptic muscarinic receptors

probably influence the release of acetylcholine by decreasing the influx of Ca^{2+} into the nerve terminals (Doležal and Tuček 1990).

The effect of several myorelaxant drugs on muscarinic receptors may be explained by their binding to an allosteric binding site on the receptors; both negative and positive cooperativities were found to occur in relations between the muscarinic and the allosteric ligand binding sites on the receptors (Nedoma *et al.* 1986, 1987, Tuček *et al.* 1990).

Excitatory amino acid receptors

NMDA, quisqualate and kainate receptors have been found to differ in the rate of their desensitization (Vyklický *et al.* 1986). The mechanism of the modulatory action of glycine at NMDA receptors has been explained by the effect of glycine on the desensitization of the receptor-channel complex (Mayer *et al.* 1989a, Vyklický jr *et al.* 1990a). The desensitization of quisqualate receptors in reduced by concanavalin A (Mayer and Vyklický jr 1989c). Kainate and quisqualate interact in their action on receptors (Vlachová *et al.* 1987). NMDA and kainate receptor channels differ in their diameters; the latter is impermeable for divalent cations (Vyklický jr *et al.* 1988). Regulatory sites for Zn^{2+} (Mayer *et al.* 1989b, Mayer and Vyklický jr 1989b) and protons (Vyklický jr *et al.* 1990b) were discovered on NMDA receptors.

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