Chapter II

Background Information

Origin and Storage of Transmitter

1. Synthesis :

The postganglionic neurons activity take up tyrosine from the extracellular fluid and by successive enzymatic reactions transform it in the neuroplasm to dopamine. The dopamine is then taken up by the storage vesicles that are formed in the cell body and that descend to the periphery (axonal flow); the uptake of dopamine and other catecholamines by these vesicles can be inhibited by drugs such as reserpine and quanethidine. In the storage vesicles, dopamine is converted to norepinephrine by dopamine β-hydroxylase which is present in both the membrane and the content of the vesicles. An increase in the neuroplasmic concentration of norepinephrine exerts a negative feedback on the activity of the enzyme tyrosine hydroxylase (end-product inhibition), whereas increased activity of the adrenergic neuron or depletion of the catecholamine stores has the opposite effect. The tyrosine hydroxylase is the rate-limiting factor in the synthesis of the adrenergic transmitter; its activity can be inhibited by drugs such as α - methyltyrosine (Vanhoutte et al.,1981).

2. Neuronal uptake :

The cell membrane of the adrenergic neuron can taken up norepinephrine and a number of other substances that can interact with adrenergic sites (neuronal uptake). This is an active process

mediated by a carrier linked to Na^+-K^+ ATPase that can be inhibited by cocaine, amphetamine derivatives and also by high concentrations of α-adrenolytic drugs such as phentolamine, phenoxybenzamine and yohimbine. Part of the norepinephrine taken up by the neuronal membrane is enzymatically destroyed by the neuronal monoamine oxidase to 3,4 - dihydroxyphenylglycol (DOPEG) before it reaches the storage sites; the DOPEG then diffuses to the extracellular space. The norepinephrine taken up by nerve endings originates either from the adrenergic terminal itself or from the blood flowing through the blood vessel.

Release of Transmitter

1. Leakage :

Part of the norepinephrine contained in the storage sites continuously diffuse to the neuroplasm and toward the extracellular space; most of it is deaminated by the neuronal monoamine oxidase to DOPEG. In isolated blood vessels incubated with [^{3}H] -norepinephrine, part to the labeled transmitter is taken up by the vascular smooth muscle cells and extraneuronal metabolites are produced; not all authors agree with this conclusion. Because of the intraneuronal deamination, the concentration of intact norepinephrine reaching the junctional cleft is usually below the threshold level required to activate the postjunctional adrenergic receptors, judged from the absence of effect of α-adrenolytic drugs on the basal tension of isolated blood vessels; one known exception is the isolated tail artery of the rat (Webb et al., 1980). Organic solvents such as propylene glycol and acetaldehyde and high concentrations of drugs

such as reserpine, the local anesthetic agent, etidocaine, the α-adrenolytic drugs prazosin and yohimbine and the combined α-and β-adrenergic blocker labetalol cause an increase in the efflux of [^{3}H]-norepinephrine and [^{3}H] DOPEG in unstimulated isolated blood vessels; inhibitors of monoamine oxidase convert the overflow of deaminated metabolites into intact [^{3}H] norepinephrine. These results suggest that these agents increase the permeability of the storage vesicles and favor the leakage of stored transmitter; in particular, if monoamine oxidase is inhibited, enough intact norepinephrine reaches the synaptic cleft to activate the postjunctional adrenergic receptor (Anderson et al.,1979).

2. Pharmacological displacement :

A number of substances, including amphetamine, butalamine, ephedrine, guanethidine and tyramine, are taken up in succession by the neuronal uptake carrier and by the vesicular membrane carrier. Presumably because they have a greater affinity for the storage proteins than the adrenergic transmitter itself, they displace norepinephrine toward the neuroplasm and extracellular space (indirect sympathomimetic effect). An important fraction of the displaced transmitter is deaminated to DOPEG by the neuronal monoamine oxidase and reaches the junctional cleft in this inactive form. Because large amounts of transmitter are displaced, however, and because the indirect sympathomimetic amine (in particular tyramine) compete for monoamine oxidase, the junctional concentration of norepinephrine can increase so that the postjunctional adrenergic receptors are activated. Unlike the exocytosis process, the pharmacological

displacement of stored norepinephrine dose not require an increase in neuroplasmic Ca^{2+} (George& Leach, 1973;) The effects of indirect sympathomimetic amines are depressed by procedures that cause denervation or catecholamine depletion, by those that inhibit the neuronal uptake process, and by α - adrenolytic drugs; by contract, they are augmented by procedures that reduce the activity of monoamine oxidase. Since the neuronal uptake carrier has a higher affinity for indirect sympathomimetic amines than for norepinephrine, the former inhibit the neuronal uptake of the latter (Hedqvist et al., 1968). Part of the response to indirect sympathomimetic amines can be ascribed to direct influences on postjunctional α - adrenergic receptors on the smooth muscle cells (Campbell & Farmer, 1968; Furchgott et al.,1963; Krishnamurty & Grollman, 1972.)

3. Exocytotic release :

The active release of norepinephrine is initiated by the active potentials generated in the ganglionic cell body. Action potentials are caused by penetration of the prejunctional membrane by Na^{+} ions together with an inwardly directed Ca^{2+} current. Because of the increased intraneuronal Ca^{2+} concentration, the storage vesicles migrate toward and fuse with the neuronal cell membrane. When the site of fusion ruptures, the vesicular contents, mainly norepinephrine and dopamine β -hydroxylase, are released in the junctional cleft. In the intact organism activation of the sympathetic nerve can be reflexly induced or caused by electrical stimulation of these nerves. Stimulation of the sympathetic nerves serving peripheral vessels results in an augmented overflow of norepinephrine in the venous

effluent from the vascular bed; the vasomotor response and the evoked release of norepinephrine disappear after degeneration of the adrenergic nerve ending, after catecholamine depletion, and after administration of inhibitors of postganglionic conduction. Similar results have been obtained in isolated perfused organ such as the kidney, the pancrease and the spleen (Bacq et al.,1974; Garcia et al., 1976; Hedqvist, 1979).

Adrenergic receptors

Adrenergic receptors were initially classified by Ahlquist (1948) as alpha and beta according to hemodynamic effects of different catecholamines. The beta adrenergic receptors were later subdivided into β_1 and β_2 on the basis of organ selectivity of a spectrum of beta-agonist (Lands et al.,1967). For α - adrenergic receptors, α_1 receptors are generally postsynaptic and α_2 receptors are generally presynaptic sites that inhibit NE release (Berthelsen & Pettinger, 1977; Starke, 1977). In addition to postsynaptic β_1 and β_2 -adrenergic receptors, some β_2 presynaptic receptors may act to facilitate NE release (Starke, 1977). Thus, the net effect of infusing epinephrine, for example, into the renal artery may involve the summation of α_1, α_2, β_1 and β_2 components at various sites in the kidney. This multiplicity of receptors and their subtypes-contributes to the currently imprecise definition of the physiological role of catecholamines in regulatory renal function.

Renal hemodynamic

Using the fluoresence histochemical technique of Falck and

Hillarp for catecholamine (1962) adrenergic nerves have been shown on the renal artery and its branches up to the afferent arteriole, the efferent arteriole, vasa recta, large veins, as well as in peritubular and juxtaglomerular locations in several mammallian species (Muller & Barajas, 1972). Catecholamines regulate renal hemodynamics predominantly via α - adrenergic mediated vasoconstriction [perhaps by α_1-receptors (Drew and Whiting, 1979)] and increased renal vascular resistance (Ahlquist, 1948). In experiment found that renal nerve stimulation (RNS) caused vasoconstriction in both cortex and medulla. This constriction was abolished or reduced by prazosin (1.5 umole/kg I.V.). These results are in agreement with the well established concept that RNS leads to renal vasoconstriction, mediated by the release of noradrenaline from sympathetic nerve endings within the kidney, and noradrenaline action on α -adrenergic receptors (Chapman et al,, 1981). Yamaguchi and Kopin (1980) have provided evidence that α_1-adrenoceptors are located near the neuroeffector junction, and respond preferentially to norepinephrine (NE) released from sympathetic nerve terminals, whereas α_2-adrenoceptors are located extrajunctionally and respond primarity to circulating catecholamines.

The administration of lethal dose of endotoxin in the intact dogs results in impaired renal function which may be attributed to the effects of systemic hypotension, renal vasoconstriction, and a possible action of endotoxin on renal tubular activity (Hinshow et al,,1961). The response of the dogs was characterized by an immediate sustained rise in angiotensin levels and a later variable rise in catecholamine levels (Hall and Hodge, 1970). In addition, in the

rabbit, norepinephrine levels in the experimental animals rose at the first hour and were statistically elevated at 5 hours (Heiffer et al., 1958).

Renal effects of Russell's viper venom

The kidney is one of the organs frequently affected in snake bites. It is well known that acute renal failure (ARF) is an important cause of death in patients who survived the early effects of a severe viper bite (Aung-Khin, 1978). A broad spectrum of renal lesions including tubular necrosis (Sitprija et al, 1974; Sitprija and Boonpucknavig, 1977), cortical necrosis (Chugh et al., 1975), glomerulonephritis (Sitprija and Boonpucknavig, 1980), interstitial inflammation, edema and hemorrhage (Sarangi et al.,1980), and acute interstitial nephritis (Sitprija et al.,1982) have been reported. The clinical syndromes which are associated with Russell's viper bite are variable. While minor reactions consist only of local pain and swelling, severe reactions may include hemorrhage, hypotension, shock, ARF and death. Diffuse fine granular deposition of IgM and the third component of complement (C_3) in mesangial areas with extension along the capillary wall is detected. By electron microscopy, occasional narrowing of the glomerular capillary lumen is observed. This is due to mesangial hyperplasia with an increase in the amount of basement membrane like matrix and the swelling of the attenuate portion of the endothelial cytoplasm. The arterial lesions are seen strikingly in Russell's viper bite. The most obvious alteration is necrotizing

arteritis of the interiobular arteries. Tubular necrosis, arteritis and thrombophlebitis of the arcuate vein are present (Sitprija et al., 1974, 1985, Chugh et al., 1978). Tubulointerstitial lesions are demonstrated by the Puchtler-Sweat method, there are hemoglobin casts in the lumen of distal convoluted tubules and collecting tubule (Sitprija et al.,1985). Necrosis of the renal cortex has been observed following the bite of Russell's viper (Chugh et al., 1975).

By immunofluorescent study deposition of β -1C globulin was noted in the arterial lesion, the glomerular mesangium, and the arteriolar wall. No immunoglobulines were noted in the lesion. Deposition of complement in the arterial lesion without immunoglobulins suggests the nonimmunologic activation of the complement system through the alternate pathway and the viper venom might be the activator. The venom might have the injurious effect to the artery, and this injurious effect was mediated through complement activation (Sitprija et al.,,1974).

Sarangi et al.,(1980) indicated that after Russell's viper bite the most common type of renal function was a reduction in urinary output with an incidence of hematuria. It is generally accepted that changes in renal functions are associated with changes on cardiovascular system. Circulatory shock may lead to ARF which is caused by filtration failure due to reduced renal circulation. Many investigators have been studied the effect of Russell's viper venom on renal hemodynamic and cardiovascular system (Chaiyabutr et al, 1984; Tongvanchai, 1984; Tungthanathanich et al.,1986). They showed that the venom caused an obviously decrease in general circulation and renal hemodynamic following the initiate of envenomation. Thereafter

the blood pressure and heart rate gradually increased and approached the control level within 2 hours. However, the rate of blood flow through the kidney, glomerular filtration rate and renal fraction (% C.O.) were decreased throughout the period of 2 to 48 hours after given a minimal lethal dose of 0.1 mg/kg of Russell's viper venom (Tungthanathanich et al.,1986). This is due to local vasoconstrictor in the kidney which is associated with an increase in renal vascular resistance. The mechanisim of renal hormonal interactions involved in intrarenal vasoconstriction are probably complex. Direct evidence for the involvement of renin-angiotensin system in responce to renal vasoconstriction after envenomation has been proposed by Chaiyabutr et al (1985) in pretreated envenomized rats with intrarenal angiotensin II blockage (MK-422, enalapril maleate). MK-422 was found to increase renal blood flow, glomerular filtration and decrease the renal vascular resistance in animals given Russell's viper venom.

According to current concepts, renal circulation regulated by two hormones system. Vasoconstriction, mediated by norephinephrine and/or the renin-angiotensin system (RAS); while postaglandin compounds and the kallikrein-kinin system act as vasodilators. Furthermore, Huang (1984) demonstrated that phospholipase A_2 (PLA_2) from vipers russelli snake venom induced an increased in plasma PGI_2 and TXA_2 level in normotensive rats. The previous study showed that imidazole (IMID), a selective thromboxane (TX) inhibitor, improves C_{in} C_{PAH}, FE_{Na} and concentrating ability of the postobstructed kidney. TX may be responsible for the persistant reductions of C_{in} and C_{PAH} in the postobstructed kidney (Cadnapaphoruchai et al.,1982).

Therefore both RAS and TXA_2 are vasoactive hormone and substance to regulate renal hemodynamic and renal function. It is believed that these substance may contribute to the alteration in envenomated kidney. In addition, the administration of lethal dose of endotoxin in dogs results in impaired renal function (Hinshow et al., 1961). The response of the dog in envenomation showed that not only sustained rise in angiotensin levels but also catecholamine levels (Hall and Hodge, 1970). Therefore, the envenomated animal which showed a decrease in renal hemodynamic and renal function may be caused by catecholamine.