preparation has been shown to be extremely sensitive to A II but not to A I[9]. A I and A II solutions were made from their stock solutions in 0.1 N acetic acid (100 µg/ml). 4 different concentrations of A I were prepared (1, 2, 4, 8 µg/ml) then crude kallikrein (Padutin®, Bayer), which was freshly diluted in saline, incubated with A I solution having a final concentration of 0.1 U/ml. These solutions were kept at 37 °C for a period of 30 min at pH 7.4. The same procedure was repeated in another series of experiments by adding SQ 20881 at the concentrations of 50 to 100 ng/ml to A I solutions before incubating with pure kallikrein. The same experimental design was followed with pure kallikrein (Kallikrein® KZC 1/75, Bayer AG). The dose-response curves of A I and A II alone, A I incubated with crude and pure kallikrein and A I incubated with crude and pure kallikrein containing SQ 20881 were determined in the aortic strips.

Results. The low concentrations of A II and relatively higher concentrations of A I (above 40 ng/ml) induced a dose-dependent contraction when superfused over the rabbit aortic strips. The contractile effect of A I was found to be potentiated after preincubation with crude kallikrein. No potentiation was observed after incubation of A I with pure kallikrein. Crude kallikrein itself neither potentiated nor relaxed when relatively higher concentrations were superfused over the rabbit aortic strips. Nonapeptide SQ 20881, which is a potent inhibitor of converting enzyme causes a significant inhibition in the response of aortic strips to A I preincubated with crude kallikrein. This inhibition was obtained when SQ 20881 was added to the medium before incubation of crude kallikrein. The degree of the inhibition by SQ 20881 was found to be almost the same when nonapeptide was used at the dose range of 10 to 100 ng/ml. Under the same experimental conditions, preincubation of SQ 20881 with A I alone did not influence the contractile response to decapeptide. The calculated results are shown in the table.

Discussion. The results of the present study clearly indicate that crude kallikrein, but not pure kallikrein, when incubated with A I causes a definite potentiation in the response of rabbit aorta. This potentiation is probably due to the conversion of A I to A II by crude kallikrein. This assumption has been based upon the following findings: 1. A I preincubated with crude kallikrein induces a myotropic activity which is equal to that obtained with A II on the aortic strip. No other fragments of angiotensin-peptides described having higher or equal myotropic activity when compared with the effect of parent peptide in the aortic strips[3]. 2. Converting enzyme inhibitor, nonapeptide SQ 20881, inhibits the potentiation of A I preincubated with crude kallikrein. In order to measure the quantity of the conversion of A I to A II by crude kallikrein, the dose-response curve of A II in each aortic strips was determined, and it indicated that almost 100% conversion occurred after preincubation of decapeptide with crude kallikrein. Since pure kallikrein did not cause a potentiation on the myotropic effect of A I, it is therefore obvious that this potentiation is not due to kallikrein itself in Padutin. It is highly possible that crude kallikrein preparations contains a kininase fraction which causes the conversion of A I to A II. The presence of a carboxypeptidase B in crude kallikrein preparations has been described previously[10].

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Neuronal accumulation and metabolism of ^{3}H-l-norepinephrine in rat portal vein: Evidence in relation to possible uneven alpha receptor distribution[1]

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Summary. The rate of accumulation and metabolism of ^{3}H-1-norepinephrine in the neuronal plexus of rat portal vein produces a small transmitter concentration gradient across the longitudinal smooth muscle layer which cannot account for the prejunctional supersensitivity observed and suggests localization of the alpha-adrenergic receptors adjacent to the nerve plexus.

The rat portal vein consists of 2 smooth muscle layers; an inner circular layer (10–20 µm thick) and an outer longitudinal layer (50–70 µm thick)[2]. A two dimensional adrenergic network is located between the smooth muscle layers[2,3]. Prejunctional hypersensitivity of the longitudinal muscle to norepinephrine (NE) applied exogenously has been demonstrated in vitro in chronically denervated and cocaine-treated tissues[2] amounting to a 13–30fold parallel shift of the dose-response curves. Because of the dimensions of the hypersensitivity in this vessel and the unlikelihood that the adrenergic nerve plexus could effectively reduce the concentration of NE throughout the longitudinal smooth muscle layer sufficiently to account for the shift in the dose-response curve, it has been postulated that the alpha-adrenergic receptors are located on the longitudinal smooth muscle cell layer directly adjacent to the nerve plexus[2,3,4,9].

In the present experiments, the rate of transport of ^{3}H-1-NE into the neuronal plexus was obtained from the neuronal accumulation and metabolism of ^{3}H-1-NE. It is self evident that NE must enter the tissue from its tissue bath at a rate equal to its rate of entry into the neuronal plexus at the steady state. From this data it was possible to calculate the necessary transmitter concentration gradient from the tissue bath to the perisynaptic region[5] to effect such a rate of entry. Since this calculation showed

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that elimination of the calculated NE concentration gradient within the muscle layer by denervation or NE neuronal uptake blockade would not account for the observed hypersensitivity to NE, it must be concluded that the major NE gradient occurs within the intrasynaptic space. In this tissue the synaptic cleft is approximately 1000 Å in width. Thus, in order to account for the observed degree of hypersensitivity the alpha-receptors are probably predominantly within this region.

Materials and methods. Approximately 10 mm of portal vein from male Sprague-Dawley rats (220–250 g) was removed, cleared of excess connective tissue and placed in a 5.0 ml Krebs-bicarbonate incubation bath at 37.5 °C bubbled with 95% O_2 and 5% CO_2. The tissues were preincubated in plain Krebs for about 45 min and incubated in ^{3}H-1-NE (2×10^{-7} M) from 10 to 60 min. Cocaine (10^{-4} M) was added to some tissues 15 min prior to the addition of ^{3}H-1-NE. The tissues and aliquots of the Krebs incubation medium were then assayed for ^{3}H-1-NE and ^{3}H-metabolites by the use of cation-exchange paper chromatography[6].

Calculation of the perisynaptic transmitter concentration was made using the diffusion equation for movement of a substance through a flat tissue layer[5]:

$$^2/_7\,M = \frac{kA\,(C_b - C_p)\,t}{d}$$

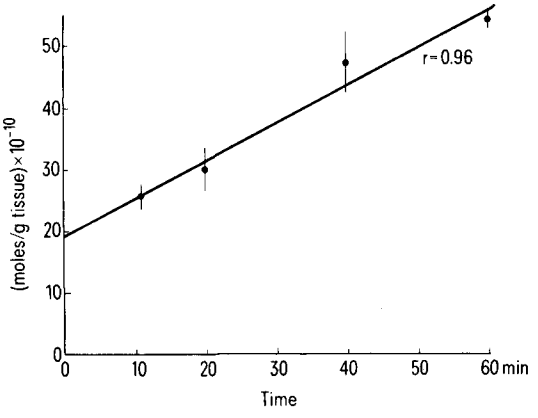
where:

M = Mass of the diffusing substance transported into the neuronal plexus in time t = 20 min
= 60.1×10^{-10} moles NE

A = Area of a plane sheet of tissue across which M diffuses
$= \frac{\text{mass/specific gravity}}{\text{thickness}}$
$= \frac{1\text{ g}/1.05\text{ g/cm}^3}{0.007\text{ cm}}$
= 136 cm²

d = Thickness of the tissue
= 0.007 cm

$(C_b - C_p)$ = Concentration gradient established by the rate of entry of NE into the neuronal plexus
C_b = Concentration of ^{3}H-1-NE in the bath
C_p = The perisynaptic concentration of ^{3}H-1-NE
k = Diffusion coefficient
= 90×10^{-6} cm²/min



^{3}H-1-norepinephrine accumulation in rat portal vein with footnote: [^{3}H-1-norepinephrine] = 2×10^{-7} M.

The rate of cocaine-sensitive accumulation was calculated by the following equation:

$$R = \frac{T_{NE_{40}} - T_{NE_{20}}}{20\text{ min}}$$

where:

R = rate of cocaine-sensitive transport of NE
$T_{NE_{40}}$ = Amount of ^{3}H-1-NE transported across the neuronal membrane after a 40 min incubation in 2×10^{-7} M ^{3}H-1-NE
$T_{NE_{20}}$ = Same as above for a 20 min incubation

[7-^{3}H-1-NE] was obtained from New England Nuclear Corp. 1-NE was obtained from Calbiochem and normetanephrine, 3,4-dihydroxyphenylglycol, 3,4-dihydroxymandelic acid, 3-methoxy-4-hydroxyphenylglycol, and 3-methoxy-4-hydroxymandelic acid from Regis Chemical Co. Cocaine was obtained from the UCLA Pharmacy. NCS tissue solubilizer was purchased from Amersham-Searle.

Results and discussion. The accumulation of unchanged ^{3}H-1-NE by the rat portal vein was linear between 20 and 40 min (figure). In order to estimate more accurately the transport of ^{3}H-1-NE across the neuronal membrane at these time points, it was necessary to monitor the neuronal accumulation and metabolism of ^{3}H-1-NE. This data for control and cocaine-treated tissues after 20 and 40 min time exposure is shown in the table. The cocaine treated tissues were used as blanks for non-neuronal accumulation and metabolism of ^{3}H-1-NE.

Having determined the rate of cocaine-sensitive ^{3}H-1-NE uptake (table), it was possible to calculate the perisynaptic concentration of NE (C_p) and the transmitter concentration gradient between the bath and the perisynaptic region ($C_b - C_p$). C_p was equal to 1.50×10^{-7} M and ($C_b - C_p$) was therefore 0.50×10^{-7} M. 2 assumptions are inherent in this calculation; homogeneity of the diffusing medium and the uniform distribution of nerve terminals throughout a twodimensional, planar nerve plexus. Török and Bevan[12] measured the entry of ^{3}H-NE into rabbit aortic media and concluded that this tissue layer was relatively homogeneous. The nerve plexus, however, forms an irregular network and the calculated concentration gradient is thus an average and not an absolute value. It should be noted that although the adrenergic plexus along the adventitial border does accumulate NE[3], the main neuronal plexus between the smooth muscle layers dominates quantitatively[13].

If the alpha-receptors were located throughout the longitudinal smooth muscle layer, then neuronal uptake would have to reduce the mean concentration of NE in the layer to more than $^1/_{13}$th of the bath concentration. Anything less could not account for the 13fold shift of the dose-response curve with chronic denervation. It must be presumed, therefore, that the site of the concentration gradient caused by neuronal uptake which would account for such a level of hypersensitivity must be within the

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synaptic cleft, i.e. within the intrasynaptic region. There is experimental evidence that such a gradient does in fact exist in this tissue during neurogenic activity[5]. This suggests that only the smooth muscle cells directly adjacent to the nerve plexus and possibly only the post-synaptic membrane contain alphareceptors.

Accumulation and metabolism of ^{3}H-1-norepinephrine in rat portal vein*

Condition	Incubation time (min)	^{3}H-1-NE + ^{3}H-metabolites** (moles/g tiss) $\times 10^{-10}$ (mean ± SE [n])
a) Control	20	87.1 ± 5.0 (5)
b) Cocaine (10^{-4} M)	20	35.6 ± 4.1 (5)
Cocaine-sensitive (a–b)	20	49.3 ± 2.0 (5)
c) Control	40	200.3 ± 14.1 (5)
d) Cocaine (10^{-4} M)	40	87.9 ± 9.9 (5)
Cocaine-sensitive (c–d)	40	112.3 ± 6.7 (5)
		(moles/g min) $\times 10^{-10}$
Rate of cocaine sensitive accumulation and metabolism of ^{3}H-1-NE		3.15 ± 0.33

* Portal veins incubated in ^{3}H-1-NE (2×10^{-7} M); tissues assayed for ^{3}H-1-NE and ^{3}H-metabolites; Krebs medium assayed for ^{3}H-metabolites.

** Metabolites: 3,4-dihydroxyphenylglycol, 3-methoxy-4-hydroxyphenylglycol, 3,4-dihydroxymandelic acid, 3-methoxy-4-hydroxymandelic acid, normetanephrine.

Recently, Aprigliano et al.[10,11] have reported only a 1.6fold shift of the contractile dose-response curve of rat portal vein to exogenous NE after treatment in vitro with 6-hydroxydopamine. Hypersensitivity of this order is commonly seen with other blood vessel strips, and implies a more homogeneous distribution of the alpha-receptors throughout the longitudinal smooth muscle layer. This value is consistent with the dimensions of the calculated intra-mural NE gradient found in this study. However, it has also been shown[4,7,8] that the latency of onset of the contractile response to exogenous NE diffusing in from either the intimal or adventitial border is consistent with alpha-receptor localization near the adrenergic nerve plexus and between the smooth muscle layers. In addition, the portal vein longitudinal smooth muscle is capable of myogenic conduction which could effectively excite smooth muscle cells throughout the longitudinal muscle layer from layers near the nerve plexus. Its contractile activity is usually dominated by pacemaker cells and the concentration of neurogenic NE outside the synaptic cleft is probably too low to be biologically effective during physiological rates of sympathetic activity[5]. Thus, the tissue seems to possess a true neuroeffector junction. In other tissues with such a junction there is restriction of the receptors to the post-synaptic membrane.

In conclusion, evidence has been derived from studies of neuronal uptake and metabolism of ^{3}H-1-NE that the concentration gradient of exogenous NE in the longitudinal muscle of the rat portal vein is not sufficient to account for a high level of denervation or prejunctional hypersensitivity. If this, in fact, occurs in this tissue, it must be the consequence of a high intrasynaptic concentration gradient and a restriction of the alpha-adrenergic receptors to this site.

Differential effects of opiates on the incorporation of [^{14}C] thiamine in the central nervous system of the rat

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Summary. Opiate agonist (morphine), pure antagonist (naloxone), mixed agonist-antagonist (nalorphine) and analgesically inactive enantiomorph (dextrorphan) produced differential stereoselective effects on the incorporation of [^{14}C] thiamine in the central nervous system of the rats. The possible role of thiamine in opiate effects and its implications are discussed.

Thiamine and its phosphorylated esters appear to play an important biophysical role in nerve conduction and excitation at the molecular level[2,3], quite independently of their well-known coenzymatic activity in the decarboxylation of α-keto acids and 2 transketolation steps of the pentose phosphate pathway. The specific location of thiamine in the axonal membrane, rather than in the cytoplasm as in other cells, its ability to form complexes with sodium and calcium ions and some neurotransmitters, its release from several neural tissues on electrical stimulation and by drugs which interact with nerve membranes, appear consistent with its participation in neural excitation and transmembrane ion transport involving permeability changes at the sodium channel[4]. In view of the involvement of approximately half of the cerebral thiamine in pyruvate oxidation and the remaining in other metabolic processes in brain, there is little if any excess of thiamine in the CNS[5] and disturbance of function in the CNS with its moderate depletion is understandable.

This study demonstrates that in chronically-morphinised rats, a highly significant decrease as compared to the controls occurred in the incorporation of [thiazole-2-^{14}C]thiamine in the brain stem; concurrent administration of naloxone, the pure narcotic antagonist with morphine abolished the highly significant increase in the incorporation of thiamine radioactivity in cortical hemispheres, cerebellum, brain stem and plasma of rats acutely-treated with a 10 mg/kg s.c. dose of morphine; dextrorphan, an analgesically-inactive morphinan did not produce any significant change in incorporation of thiamine radioactivity in these areas of the CNS and plasma; naloxone produced a significant decrease in the incorporation of thiamine radioactivity only in the brain