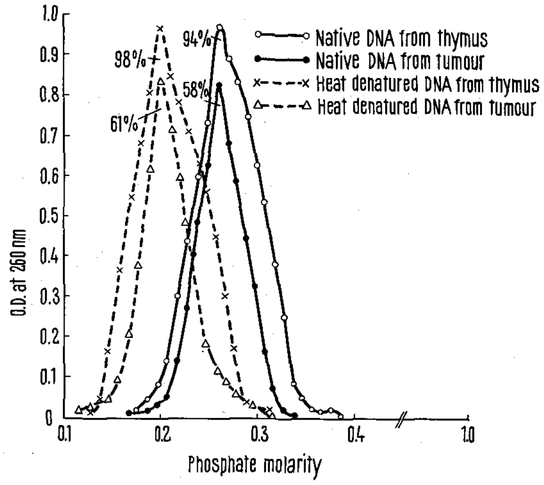
On heat denaturation (100 °C, 15 min) of the DNA in tumour as well as control samples, the peaks at $0.26 M$ PO_4 shifted close to $0.2 M$ PO_4 while no significant change in the behaviour in the $1.0 M$ region was observed in both these cases.



The chromatographic elution patterns of DNA on hydroxyapatite column using phosphate buffer gradient.

The fractions eluting at $0.26 M$ PO_4 from both the present sources of DNA conform to the observations of earlier workers with native DNA from chicken erythrocyte, calf thymus, *Escherichia coli*[4], as well as T2 and λ bacteriophage[5]. The observed shift to lower PO_4 molarity of the eluent for these fractions on heat denaturation of the DNA samples also supports such a correlation and could be due to conversion of double stranded DNA into single stranded variety. On the other hand, the observation of the more adherring type of DNA in the present investigations which seems to form a significant fraction in the samples from tumour cells only, does not find easy correlation with known data. The stability of this fraction to heat treatment is also noteworthy. It would be of interest to know if this fraction showed any characteristic differences in its biological behaviour when compared with the more common native DNA fraction eluting in the PO_4 concentration range 0.2–$0.3 M$.

Zusammenfassung. Zwischen DNS normaler und neoplastischer Zellen besteht ein wesentlicher biochemischer Unterschied.

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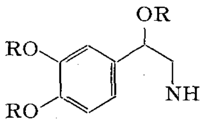
29 July 1968.*

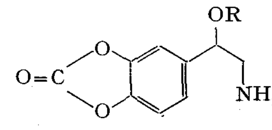
Labile Lipophilic Derivatives of Norepinephrine Capable of Crossing the Blood-Brain Barrier

One method of modifying the activity of pharmacological agents is through synthesis of labile derivatives which undergo either enzymatic or non-enzymatic hydrolysis in vivo to regenerate the parent drug[1–5]. Differences in metabolism, excretion, active or passive transport and tissue localization of these derivatives should influence greatly the onset, duration and type of activity characteristic of the parent drug. The catechol amines, (nor) epinephrine and dopamine may be modified either at the basic nitrogen or at the phenolic groups. Labile lipophilic derivatives of catechol amines would be expected to cross the 'blood-brain barrier', enter the central nervous system (CNS) and, by hydrolysis, generate the parent neurohumoral transmitters in situ. In addition, such labile derivatives of norepinephrine-^3H and other neurohumoral transmitters might provide a method of labeling central storage sites with radioactive amine for the study of amine uptake, storage, release and metabolism. Such CNS studies normally make use of radioactive metabolic precursors which readily cross the 'blood-brain barrier', for example: tyrosine[6,7], dopa[6,7], or 3,4-dihydroxyphenylserine[8], or involve the direct, intracisternal injection of the amine[9].

Our synthetic approach to 'latentiation' of norepinephrine was guided by the observation that phenolic derivatives of catecholamines, such as the 0,0-dibenzoate, 0,0-diacetate or 0,0-*bis*(ethylcarbonate), caused release of norepinephrine-^3H from cardiac tissue in 2 h[10], a clear indication that rapid hydrolysis of these esters to the active catechols must have occurred in vivo.



I



III

I: R = Ac
II: R = $(CH_3)_3Si$

Two lipophilic derivatives of norepinephrine were prepared. One, 3,4,β-triacetyl-L-norepinephrine (I) was prepared by acetylation of *N*-carbobenzyloxy-L-norepine-

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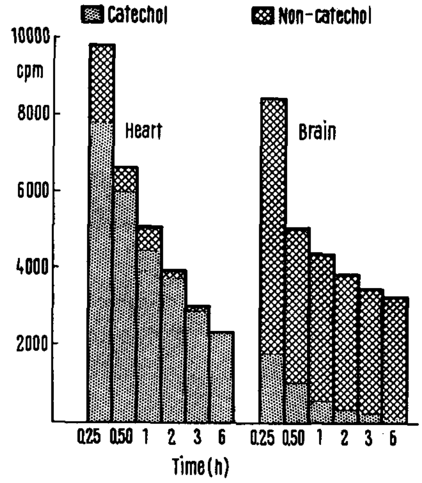
phrine with acetic anhydride-pyridine followed by reductive removal of the carbobenzyloxy group. The amorphous triacetate was homogeneous by thin-layer chromatography (Silica gel, *n*-butanol; acetic acid, water, 5:3:1, Rf 0.55) while NMR-spectroscopy and high resolution mass spectrometry confirmed structure I. Analogously, *N*-carbobenzyloxy-L-norepinephrine reacted with phosgene and pyridine to give, after catalytic decarbobenzylation, the cyclic carbonate of L-norepinephrine (III).

Compounds I and III, when tested for their effect on the release of cardiac norepinephrine-^{3}H[10–14], caused significant release of norepinephrine-^{3}H from heart during the assay period of 2 h. In addition, local piloerection at the site of s.c. injection (administered as solution in 50% dimethylsulfoxide) was observed. Compounds I and III had an ED_{50} of 2.7 and 5.2 mg/kg, respectively, for cardiac norepinephrine-^{3}H release. L-Norepinephrine, itself, has an ED_{50} of < 1.2 mg/kg.

Distribution of radioactivity between brain and heart after administration of norepinephrine-^{3}H, 3,4,β-triacetylnorepinephrine-^{3}H and 3,4,β-*Tris*(trimethylsilyl)-norepinephrine-^{3}H

Compound[a]	Total activity injected (nc)	Activity in heart (nc)	Activity in brain (nc)	Ratio heart/brain
Norepinephrine-^{3}H	104	7.3	0.4	18.3
3,4,β-Triacetyl-norepinephrine-^{3}H	10	0.07	0.05	1.2
3,4,β-*Tris*(trimethyl-silyl)-norepinephrine-^{3}H	13	0.06	0.10	0.60

[a] Norepinephrine-7-^{3}H and derivatives were administered to mice (male, 20 g) by tail vein injection 30 min prior to sacrifice. Results are expressed as the mean values of the total activity/organ from 10 animals. Activity was measured by liquid scintillation spectrophotometry. Counting efficiency 5–14%.



3,4,β-Triacetylnorepinephrine (0.5 µc) was administered i.v. (tail vein) to 20-g male mice. At the times indicated 5 animals were sacrificed, tissues removed, homogenized in 0.4*N* perchloric acid. Following centrifugation the supernatant fluid was separated into catechol and non-catechol fractions by the alumina adsorption technique. Values are reported as activity (cpm corrected to 10% efficiency) per total organ.

To ascertain whether such derivatives would be practical for labeling brain norepinephrine stores with tracer amounts of norepinephrine-^{3}H, the 3,4,β-triacetate I was prepared from norepinephrine-7-^{3}H (New England Nuclear Corp., 5 µc/nmole). In addition, the 3,4,β-*Tris*(trimethylsilyl) derivative (II) of norepinephrine-7-^{3}H (5 µc/m*M*) was synthesized by a modification of the procedure of Kawai and Tamura[15] in which pyridine-hexamethyldisilazane-trimethylsilyl chloride (10:2:1) was used as silylating reagent. Gas chromatography and mass spectrometry confirmed the structure as the 3,4,β-*Tris*(trimethylsilyl)-norepinephrine (mol. wt. 388, loss of CH_2–NH_2). Tracer amounts of these derivatives (I and II) and of norepinephrine-7-^{3}H, itself, were administered i.v. to mice in a mixture (0.1 ml) of dimethylsulfoxide and isotonic sodium chloride (11:1). In contrast to the pattern of distribution obtained with free norepinephrine both derivatives readily entered the CNS (Table). The technique did not seem practical for labeling central stores of norepinephrine, since both lipophilic derivatives survived for long periods in the brain, presumably in fatty tissue. The disappearance of 3,4,β-triacetylnorepinephrine-7-^{3}H in brain and heart is shown in the Figure. The turnover of norepinephrine-^{3}H in brain after intracisternal administration may be influenced by various drugs[9], but similar attempts to influence the turnover of norepinephrine-^{3}H formed from the latent precursors were rather unsuccessful. This may be due to the formation of norepinephrine-^{3}H from the precursor both continuously in situ and also during isolation. However, on the basis of the observations reported here, compounds I, II, III or related lipophilic derivatives might be useful in providing the CNS with a sustained release of the neurotransmitter norepinephrine.

Zusammenfassung. Das in vivo leicht hydrolysierbare 0,0,0-Triacetat und das zyklische Brenzkatechin-carbonat des L-Noradrenalins setzen im Herzen der Maus tritiiertes Noradrenalin frei. Beide Derivate sowie das 0,0,0-Trimethylsilyl-L-noradrenalin-7-^{3}H, sind genügend lipophil, um leicht die Liquorschranke zu überschreiten, wo sie im Zentralnervensystem langsam tritiiertes Noradrenalin freisetzen. Solche Noradrenalin-Depot-Derivate sind möglicherweise von therapeutischem Interesse.

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