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NORMETANEPHRINE AND OCTOPAMINE INVOLVEMENT IN NORMACROMERINE BIOSYNTHESIS IN *CORYPHANTHA MACROMERIS* VAR. *RUNYONII*

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Key Word Index—*Coryphantha macromeris* var. *runyonii*; Cactaceae; biosynthesis; phenethylamines; normacromerine; normetanephrine; octopamine.

Abstract—Administration of [7-^{3}H]normetanephrine to living *Coryphantha macromeris* var. *runyonii* resulted in the formation of labeled normacromerine (*N*-methyl-3,4-dimethoxy-β-hydroxyphenethylamine). Trapping experiments were employed to demonstrate the natural occurrence of normetanephrine in the cactus and its formation from administered [7-^{14}C]norepinephrine. [2-^{3}H]Octopamine was found to be a very inefficient precursor to the psychoactive normacromerine and therefore is apparently not involved with the catecholamine metabolism in *C. macromeris* var. *runyonii*.

INTRODUCTION

The Dona Ana cactus, *Coryphantha macromeris* (Engelm.) Br. & R. var. *runyonii* (Br. & R.) L. Benson, is being promoted as a natural and legal psychedelic agent with about one-fifth the potency of peyote [*Lophophora williamsii* (Lem.) Coult.] [1, 2]. Like peyote, Dona Ana produces and accumulates various methylated catecholamine derivatives [3, 4]. Of these phenethylamines, normacromerine (*N*-methyl-3,4-dimethoxy-β-hydroxyphenethylamine) is by far the most abundant [4] and has been shown to affect animal behavior in such a way as to suggest psychoactivity [5]. A preliminary biosynthetic investigation demonstrated the existence of a C_6–C_2 pathway in *C. macromeris* var. *runyonii* where tyrosine is decarboxylated and the resulting tyramine is metabolized to give normacromerine [6]. Later work demonstrated the operation of catecholamine metabolism in the cactus when both epinephrine and norepinephrine were found to be precursors to normacromerine [7]. However, these studies suggested a branched pathway because norepinephrine was metabolized to normacromerine less efficiently than either tyramine or epinephrine.

The present study deals with an investigation of the norepinephrine branch of the proposed normacromerine biosynthetic pathway in *C. macromeris* var. *runyonii* as illustrated in Fig. 1. There are established biochemical precedents which support each step of the proposed sequence of the norepinephrine branch. In rats, octopamine has been established as the intermediate during the conversion of tyramine to norepinephrine [8]. The enzymatic methylation of the *meta*-hydroxyl group of norepinephrine to give normetanephrine has been well established [9] and is now generally recognized as the major catabolic reaction of norepinephrine in mammals. Metanephrine, the immediate precursor to normacromerine in the Dona Ana cactus [10], may be formed from normetanephrine under the influence of an enzyme isolated from rabbit lung [11]. In an effort to validate the postulated norepinephrine branch of the biosynthetic pathways leading to normacromerine, octopamine and normetanephrine were examined as potential precursors.

RESULTS AND DISCUSSION

A group of three living *C. macromeris* var. *runyonii* were injected at several above-ground sites with a 0.01 N acetic acid solution of DL[2-^{3}H]octopamine (1.989×10^9 dpm administered). Following a 27-day incubation period, the cacti were extracted and processed [4] to give an alkaloid fraction. The usual preparative TLC procedure [7] was employed to isolate 52 mg of normacromerine HCl (1.961×10^5 dpm) from the alkaloid mixture. Based on the total activity of octopamine administered to the cacti, less than 0.01% was recovered as crystalline normacromerine HCl. These data suggest that octopamine is not a normacromerine precursor in the Dona Ana cactus and therefore the usual chemical degradations of the isolated material were not conducted.

Three healthy *C. macromeris* var. *runyonii* specimens were injected with DL[7-^{3}H]normetanephrine dissolved in 0.01 N acetic acid (2.070×10^9 dpm administered) and, after 24 days of incubation, were processed in the usual manner [4, 7] to give 240 mg of normacromerine HCl (8.478×10^6 dpm). Therefore 0.41% of the radioactivity of the administered normetanephrine was associated with the isolated normacromerine. In order to detect possible contamination and/or randomization, the radioactive normacromerine HCl was oxidized with sodium periodate as previously described [10]. The veratraldehyde semicarbazone from this degradation was found to contain over 96% of the original radioactivity associated with normacromerine HCl while the formaldemethone contained less than 3%. This chemical degradation demonstrated that the ^{3}H label was specifically associated with the β-position of the isolated normacromerine.

Since normetanephrine has never been detected in extracts of *C. macromeris* var. *runyonii*, it was essential to demonstrate whether this compound was a natural or an

aberrant precursor to normacromerine. This task was accomplished by using the inverse isotope dilution technique which is commonly referred to as a trapping experiment. Three Dona Ana cacti were allowed to incubate for 11 days after being injected with a 0.1 N aqueous tartaric acid solution of DL [7-^{14}C]norepinephrine (1.156×10^8 dpm administered). The treated plants were extracted by homogenizing with EtOH–HOAc (19:1) and the homogenate filtered. After adding 150 mg of DL-normetanephrine HCl to the filtrate, the usual process [4] was employed to give an alkaloid fraction. Preparative TLC of the alkaloid mixture over Si gel (1 mm) with $CHCl_3$–MeOH–1 N NH_4OH (60:35:5) afforded 88 mg normetanephrine HCl (1.324×10^5 dpm). The specific activity of the isolated normetanephrine HCl remained constant after recrystallization. The recovery of 0.11 % of the administered radioactivity demonstrated the occurrence of normetanephrine in *C. macromeris* var. *runyonii* and its formation from norepinephrine.

This work indicates that normetanephrine is a naturally occurring precursor to normacromerine while octopamine is not. Comparing precursor incorporation

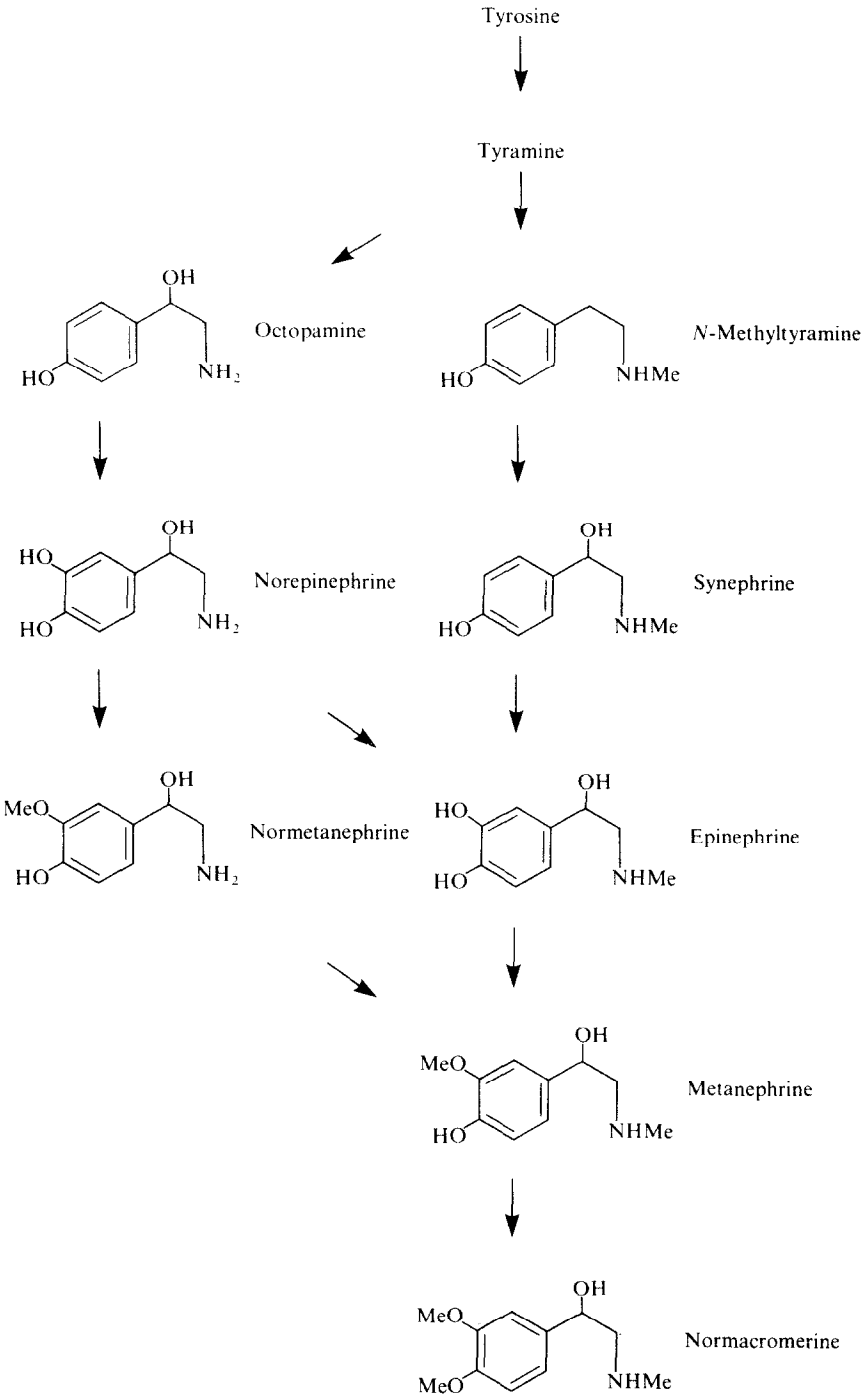

Fig. 1. Biosynthetic pathways to normacromerine.

efficiencies presented in this paper and in the past [7, 10], it appears that normetanephrine does not serve as a mainstream precursor to normacromerine but rather as a bypass intermediate between norepinephrine and metanephrine. The octopamine incorporation data from this study prompted a re-evaluation of the proposed early steps of normacromerine biosynthesis. *N*-Methyltyramine has recently been shown to be a precursor to normacromerine [12]. However, *N*-methyltyramine was metabolized to normacromerine less efficiently than tyramine, which again suggests a branched biosynthetic pathway. One branch would logically involve synephrine serving as an intermediate between *N*-methyltyramine and epinephrine. The other branch apparently does not involve octopamine serving as an intermediate between tyramine and norepinephrine. An alternative to octopamine is dopamine. The preliminary investigation of normacromerine biosynthesis revealed that dopamine was a very inefficient precursor [6]. However, during those experiments large masses of black gelatinous material were observed at the sites where the dopamine solution had been injected into the cacti. Enzymatic conversion of dopamine to melanin is well known and this process could have occurred within the plant, thereby effectively depriving the site of alkaloid biosynthesis of this precursor. This hypothesis is supported by Steelink *et al.* [13] who have demonstrated the rapid enzymatic oxidation of dopamine to melanin following injury to the cortical tissue of the giant saguaro cactus, *Carnegia gigantea* (Engelm.) Br. & R. Experiments are now in progress in this laboratory to determine the efficacy of dopamine and synephrine as normacromerine precursors in cell-free systems.

EXPERIMENTAL

Radiochemicals. DL-[7-^{3}H]Normetanephrine (sp. act. 7.9 Ci/mM) and DL-[2-^{3}H]octopamine (sp. act. 12.28 Ci/mM) were purchased (New England Nuclear Corp.)

Plant material and growing conditions. C. macromeris var. *runyonii* was purchased from Aztekakti. All cacti were watered bimonthly and were maintained in a controlled environment chamber (Scientific Systems) on a diurnal cycle of 14 hr light and 10 hr dark. The temp. was maintained at 32° during the light period and at 18° during the dark period.

Counting procedures. Triplicate samples dissolved in a scintillator consisting of 0.5% PPO and 0.05% dimethyl POPOP in toluene–*p*-dioxane (1:1) were counted in a liquid scintillation spectrometer (Beckman LS-230). All samples were counted to an error of less than ±1%. The counter efficiency was determined for each sample by the internal standard method using either [^{14}C] or [^{3}H]toluene. A blank value was obtained routinely to determine the magnitude of background radiation.

Identification of isolated phenethylamines. The identity of the isolated normetanephrine and normacromerine was established by co-chromatography and by mp determinations on the hydrochloride derivatives. The hydrochlorides were crystallized ×3 in order to establish radiochemical purity.

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