The occurrence of para-octopamine in the hypothalamus of the domestic fowl: Effects of drugs on its storage and metabolism

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Summary. The domestic fowl hypothalamus contains 2.7 ng/g of p-octopamine. These levels are significantly reduced after fusaric acid or reserpine administration or increased after pargyline or tranylcypromine.

Phylogenetic distribution studies have revealed that neural tissues of some invertebrates contain 'octopamine' (presumably the para and meta isomers). The levels of the octopamines (until recently, the isomers had not been resolved and therefore any measurement represented a combination of the two) may be as high as 8100 ng/g and neurophysiological and pharmacological studies suggested that it may function as a neurotransmitter[2]. In the mammalian brain, the levels are markedly lower (2.4 ng/g for the rat brain)[3] although its turnover (i.e. half-life) is very fast[4,5]. It appears to be stored in a reserpine-sensitive compartment[6-8]. The development of a specific and sensitive radioenzymatic-derivatisation-chromatographic procedure permits the resolution of the p- and m-isomers of octopamine[8]; it is this new method that has been used in this work.

The fact that the p- and m-isomers of tyramine are present in the domestic fowl brain[9] along with the β-hydroxylated metabolites of dopamine (noradrenaline and adrenaline)[10] suggested that the β-hydroxylated tyramines (p- and m-octopamine) also may be present. In this paper, the presence, metabolism and storage of p-octopamine in the domestic fowl hypothalamus has been studied; in addition, the presence or otherwise of m-octopamine was investigated. For comparison, some p-octopamine determinations were carried out in mouse and guinea-pig hypothalami.

Female white Leghorn domestic fowls (*Gallus domesticus*, 1.3–2.1 kg b.wt and of approximately 2.5 years of age) were killed by decapitation. The brain was removed, divided by a median sagittal section and the hypothalamus removed, frozen in dry ice and weighed (weight range 32–52 mg). Drugs were administered by i.m. injection in the breast muscle. Tranylcypromine sulfate (Smith, Kline and French), pargyline hydrochloride (Abbott) and fusaric acid (Sigma) were dissolved in saline and reserpine (Sigma) was first solubilized with 50–100 µl of glacial acetic acid and then diluted 50–100 times with isotonic glucose solution. The control animals were injected with the corresponding vehicle solution. Male albino Swiss mice (20–25 g b.wt), and male guinea-pigs (English Short Hair strain, 250 g b.wt) were killed and the hypothalamus dissected. The estimations were carried out in the pooled hypothalami of 3 mice (30–36 mg) and single guinea-pig hypothalamus (35–48 mg). Tissue homogenates were prepared in the presence of a monoamine oxidase inhibitor, which protected the octopamines from oxidative deamination, and then heated to denature proteins which were removed by centrifugation. Portions of the deproteinized supernatant corresponding to about 10 mg of tissue were then incubated with partially purified phenylethanolamine-N-methyltransferase (EC 2.1.1) and tritiated S-adenosylmethionine which served as methyl donor substance[11,12]. Internal standards were prepared by adding supplements of p- or m-octopamine to a portion of each tissue homogenate and the blanks were prepared by the addition of Tris-HCl buffer solution. The p- and m-isomers of synephrine formed in the reaction were extracted with ethylacetate, transferred to another test tube and evaporated to dryness; the residues were dissolved in sodium carbonate and 1-dimethylamino-naphthalene-5-sulfonyl chloride (dansyl chloride) added[8]. The dansylated amines were extracted and applied to the origin of a thin layer silica-gel plate (Brinkmann Instr. Ltd) and separated 1st in chloroform: butylacetate (5:2, v/v) and then after elution and application to a 2nd plate in benzene: triethylamine (12:1, v/v). After the final separation, the dansyl synephrine zones were removed from the plate, extracted and the radioactivity measured in a scintillation counter. The experimental samples contained about 20–30 pg of p-octopamine and the radioactivity produced was about 2–4 times higher than that in the reagent blanks.

The endogenous hypothalamic concentration of p-octopamine in the domestic fowl is about 2.7 ng/g of fresh tissue (table) whilst that of m-octopamine is below the limits of the sensitivity of the method (<0.5 ng/g). The administration of reserpine produced a marked decrease in the p-octopamine levels, to 13% of the control level, 3 h after the administration of 1 mg/kg. 24 h after the drug administration, the values had partially recovered to 34% of controls (table). Monoamine oxidase inhibition by either tranylcypromine (10 mg/kg) or pargyline (100 mg/kg) produced marked increases in the p-octopamine level (about 17- and 23fold increases respectively). In contrast, the level of m-octopamine remained below the limit of sensitivity of the method (<0.5 ng/g). The administration of fusaric acid (an inhibitor of dopamine-β-hydroxylase) produced a marked reduction (to 54% of controls) in the p-octopamine levels (table). The mouse hypothalamic concentration of p-octopamine was 9.00 ± 0.55 (Buck et al.[7]) and that of the guinea-pig, 1.23 ± 0.22 (Juorio[9]), (results in ng/g of fresh tissue are means±SEM, number of experiments in parentheses).

The radioenzymatic method for the determination of 'octopamine' as described by Molinoff, Landsberg and Axelrod[11], or Saavedra[12] involves the transformation of both p-octopamine and m-octopamine (if present) into p-synephrine and m-synephrine respectively, and both isomers are therefore jointly estimated as 'octopamine'. In the modification described by Danielson, Boulton and Robertson[8], the products of the radioenzymatic reaction (p-synephrine and m-synephrine) are converted into their bis-dansyl derivatives and separated by thin layer chromatography. In this way octopamine is resolved into its p- and m-isomeric constituents as well as removing any other radiolabelled contaminant. The level of p-octopamine in the hypothalamus of the domestic fowl (2.7 ng/g) is remarka-

The concentration of p-octopamine (p-OA) in the domestic fowl hypothalamus and the effect of the i.m. administration of reserpine, tranylcypromine, pargyline and fusaric acid

	Dose (mg/kg)	Time (h)	p-OA (ng/g)	% of controls
Controls	–	–	2.66± 0.46 (14)	–
Reserpine	1	3	0.34± 0.22 (4)***	13
Reserpine	1	24	0.91± 0.34 (6)**	34
Tranylcypromine	10	2.5	46.07± 7.73 (7)***	1732
Pargyline	100	3	61.86± 14.51 (5)***	2326
Fusaric acid	100	2	1.43± 0.23 (3)*	54

Values are means (± SEM, number of experiments in parentheses) in ng/g of fresh tissue. Student's t-test: * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

bly similar to that found in the hypothalamus of the rat[8], mouse or guinea-pig (3.4, 9.0 and 1.2 ng/g respectively).

Octopus ganglia contain relatively high concentrations of octopamine (1310 ng/g in the optic lobe, e.g.) and the administration of reserpine (4 mg/kg) produced a marked reduction (to about 10% of control value) in its concentration[6]. It has been reported that a similar dose of reserpine does not produce a reduction in the whole mammalian brain octopamine levels[13]; more recent work, however, has shown that reserpine (1–3 mg/kg) does indeed produce marked reductions (to 32% and 1% of controls) in the hypothalamic levels[7,8]. The present experiments show that the level of hypothalamic p-octopamine in the domestic fowl is indeed markedly reduced as a result of reserpine (1 mg/kg) administration and this suggests that the p-octopamine, or perhaps some of its precursors, are kept in a reserpine-sensitive storage compartment as has been shown to be the case in mammals for the catecholamines and tyramines[14–16].

The substantial increases in p-octopamine levels that follow after monoamine oxidase inhibition with tranylcypromine and pargyline respectively, strongly suggest that p-octopamine in the domestic fowl possesses an active turnover rate as has been shown in the case of the mammalian brain[4,5]. Dopamine-β-hydroxylase is the enzyme that catalyzes the β-hydroxylation of dopamine as well as that of p- and m-tyramine to yield respectively, noradrenaline, p-octopamine or m-octopamine[17]. Since the administration of fusaric acid (a dopamine-β-hydroxylase inhibitor) produced a marked decrease in the domestic fowl hypothalamic p-octopamine, this synthetic route would seem to be the one followed in this animal species as well.

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Prolactin secretion inhibition by a new 8α-amino-ergoline, CH 29-717

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Summary. In rats, CH 29-717 inhibits basal and physiologically or chemically stimulated prolactin secretion. It is more potent than the standard bromocriptine.

10 years ago we provided the first indirect evidence that a synthetic ergot derivative, 2-Br-α-ergokryptine-mesylate (CB 154, bromocriptine) inhibits prolactin secretion[1]. Since that time many new ergot derivatives have been found in various laboratories which also inhibit the secretion of this hormone[2]. Unfortunately, few of them have been adequately characterized[3]. We wish to report data obtained in the rat on the prolactin secretion inhibitory activity of a new synthetic ergot compound N,N-dimethyl-N'-(6-methyl-ergoline-8α-yl)-sulfamide hydrochloride, code number CH 29-717. Its effects were compared with those of bromocriptine.

In all experiments, CH 29-717 was dissolved in saline; bromocriptine was dissolved in acidified ethanol (70%) and then diluted with saline. All experiments were carried out with rats kept under a regimen of 14 h light, 10 h dark, at constant temperature and humidity. Food and water were freely available. The results reported for the 2 compounds are not from experiments run in parallel.

1. *Inhibition of ovum implantation. Method.* Adult proestrus

Table 1. Incidence (%) of milk-spots in the stomach of pups of treated and untreated lactating rats

Treatment	Dose ($mg \cdot kg^{-1} \cdot d^{-1}$ orally)	Number of nursing rats treated*	Pups showing milk-spots on day (%) 4	5	6	7	8	9	10	11
Vehicle	0	6	100	100	98	100	98	98	96	98
CH 29-717	0.0125	5	100	100	95	95	88	98	100	100
	0.0250	6	100	100	96	83	69	79	98	100
	0.0500	5	100	100	75	0	3	8	100	100
				Treatment period						

*7 or 8 pups per nursing rat.