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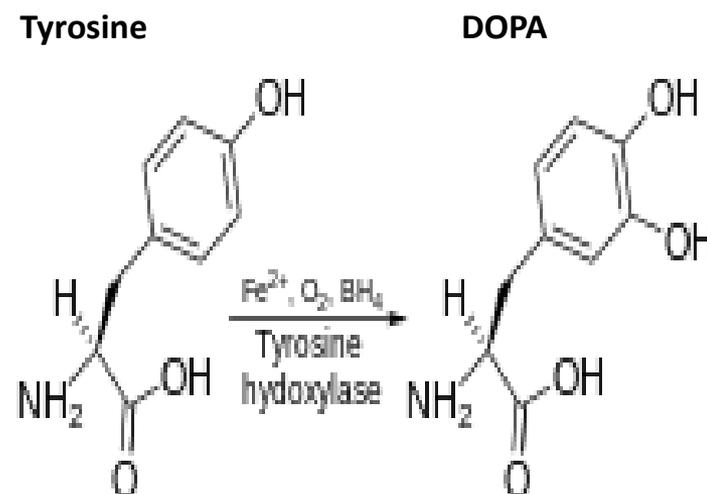
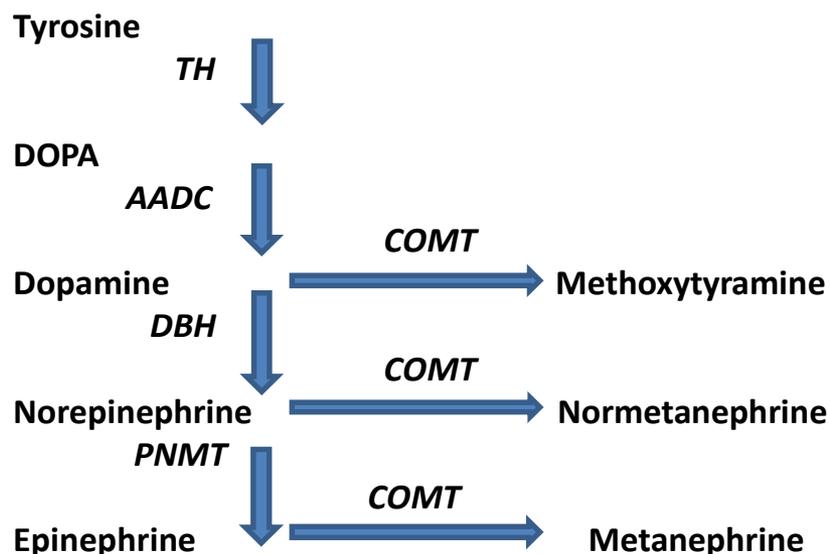
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# Tyrosine hydroxylase, the rate-limiting enzyme in catecholamine biosynthesis could be an index of functionality in pheochromocytoma diagnosis



# Abstract

- *Aim:* Excess release of catecholamines is a characteristic for pheochromocytomas. The rate of catecholamine synthesis is determined by tyrosine hydroxylase (TH) enzyme. Usually, TH is detected by immunohistochemistry. In our study, we evaluated this enzyme in plasma from pheochromocytoma subjects establishing correlations with some metabolites.
- *Subjects and Methods:* 10 subjects (9 women/1man aged 40-72 years) clinically suspected of pheochromocytoma, were biochemically investigated for free plasma normetanephrines (NMNp)/metanephrines (MNp), plasma chromogranin A (CgA), plasma TH. Comparison of tumoral metabolites values was done with our lab normal range. Statistical analysis used multiple regression to evaluate relationship between TH and all 3 parameters: NMNp/MNp/CgA.
- *Results:* All subjects showed excessive plasma NMNp (median: 1434 pg/mL); in 7 cases was an over secretion of MNp (median: 441pg/mL) and CgA was greater in 9 cases (median: 668 ng/mL). TH was identified in all plasma samples with a median of 2.08 ng/mL. TH greater values were detected in cases with an over excess of metabolites. Good correlations were established between NMNp/TH (0.51), MNp/TH (0.81) and NMNp/CgA (0.71).
- *Conclusion:* Metabolites in excess are well correlated with TH values proving a great rate of catecholamine synthesis in some cases. We could affirm TH could be used as an index of functionality in pheochromocytoma diagnosis.
- *Key words:* pheochromocytoma, tyrosine-hydroxylase, free normetanephrines, free metanephrines, chromogranin A



# Introduction

- Tyrosine hydroxylase (TH) discovered in 1964 is a tetrahydrobiopterin (BH4)-requiring monooxygenase that catalyzes the first and rate-limiting step in the biosynthesis of catecholamines: dopamine, noradrenaline, and adrenaline (*T. Nagatsu et al, J Neural Transm (Vienna) august 2016*)
- TH is soluble and present in the cytoplasm in catecholamines-producing cells (*J. C. Waymire et al., J Clin Invest vol 51,1972,1798-1804*)
- Purification of TH was difficult, but was finally achieved in early 1980's from various tissues: bovine adrenal medulla, rat adrenals, human brain and adrenals
- The primary structure of the enzyme from various species including humans was determined by cloning of cDNA after the 1980's
- TH is distributed in sympathetic ganglia, the adrenal medulla, the central nervous system and also in pheochromocytomas



# Introduction

- Pheochromocytomas contained a significantly larger amount of catecholamines and greater TH activity than did normal adrenal medullas
- In pheochromocytomas, TH activity is mainly regulated by the TH mRNA expression rather than by short-term control involving TH protein phosphorylation
- TH activity was significantly high in pheochromocytoma and paraganglioma as compared with that in normal adrenal gland, whereas TH activity was low in a neuroblastoma and was undetectable in other tumors
- These findings indicate that TH correlates well with the biosynthetic function of catecholamines in the tumor cell and both the immunostaining of TH and the measurement of its activity in adreno-medullary and related tumors may provide some information about the process of cell differentiation in these tumors. (*Iwase, K et al. J Endocrinol Invest 1994,17: 235*)
- The aim of our study was to identify the presence of TH by biochemical means in a group of pheochromocytoma cases versus a normal control group and to prove the utility of this enzyme as an index of tumoral functionality



# Results and discussion

- In this retrospective study (2010-2011), we biochemically investigated 10 patients (9 women /1 man aged 40-72 years) clinically suspected of pheochromocytoma and 10 normal subjects without endocrine dysfunction (10 women aged 30 - 60years)
- All subjects collected in the morning at 9 am (after an overnight fasting, free of drugs) a sample of plasma (into EDTA vacutainer). After centrifugation, plasma supernatants were aliquoted and stored at -20°C until assayed.
- Free plasma normetanephrines (NMNp)/ metanephrines (MNp), plasma chromogranin A (CgA) and plasma TH were tested by research Elisa methods.
- Statistical processing of data was done using MedCalc Software version 8.0.0.1



# Results and discussion

- All 10 tumoral patients showed excessive plasma NMNp with a peak of 7000pg/mL
- In 7 cases was an over secretion of MNp with a peak of 3365pg/mL
- Cg A was greater in 9 cases with a peak of 1439 ng/mL
- TH was identified in all tumoral samples with the highest value of 20 ng/mL
- TH greater values were detected in 2 cases with an over excess of NMNp/MNp and CgA
- Mean values for all 4 parameters were compared in both groups: tumor and control group by T-test
- All assayed parameters differed significantly in tumor group in comparison to control group (Table1)



# Results and discussion

- Spearman's correlation coefficients were established between NMNp/TH; MNp/TH ; CgA/TH; NMNp/CgA; MNp/CgA
- A high Spearman's coefficient were established between MNp/TH proving a great rate of metabolic conversion of normetanephrines to metanephrines
- CgA correlated well with TH proving the importance of this marker for pheochromocytoma diagnosis(Table 1)
- In Fig 1,2,3,4 we established multiple correlations by statistical regression between NMNp/TH; MNp/TH; CgA/TH and NMNp/MNp
- Good multiple correlation coefficients were obtained between NMNp/TH; Mp/TH and between NMNp/CgA



# Results and discussion

- All 10 tumoral cases were surgically excised in other units and post-surgery histopathological exam proved all of them as benign pheochromocytomas
- Our study proved excessive biosynthesis of catecholamines in all 10 cases of pheochromocytoma and also of the colocalized CgA
- TH was present in all plasma tumoral samples proving its important role in the biosynthesis of catecholamines in pheochromocytoma
- Metabolites in excess in tumoral cases are well correlated with TH values proving a great rate of catecholamine biosynthesis and also an increased rate of production of intratumoral metabolism (dependence of metanephrines production on leakage of catecholamines from vesicular stores) (*G. Eisenhofer et al., Pharmacol Rev 56:331-349,2004*)



# Results and discussion

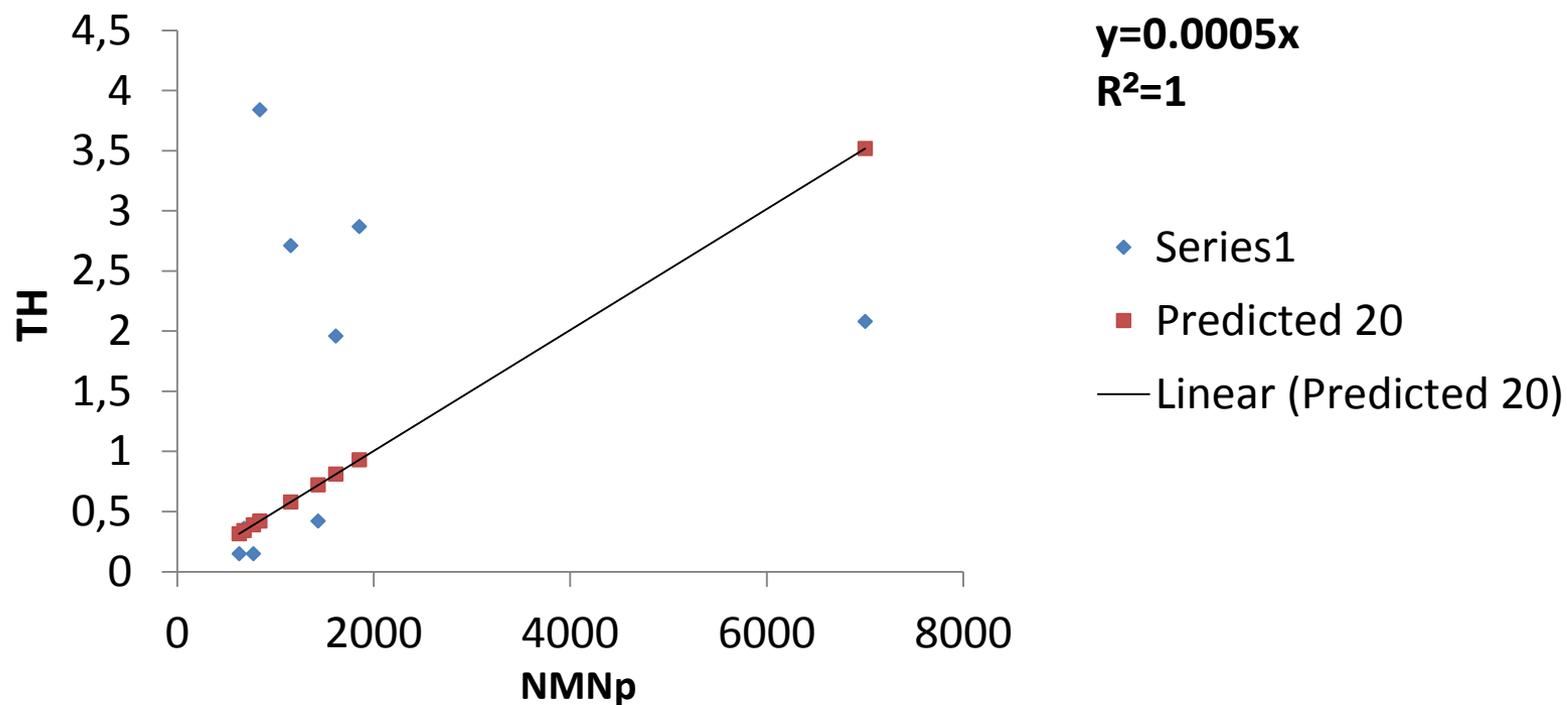
Table 1 - Plasma NMNp, MNp, CgA and TH in 10 pheochromocytomas vs. normal subjects

Cases number	NMNp pg/mL Mean/SEM Range	MNp pg/mL Mean/SEM Range	CgA ng/mL Mean/SEM Range	TH ng/mL Mean/SEM Range
10 tumors	2092.3 ± 510.96 630 - 7000	782.4 ± 236.12 34 - 3365	764.7 ± 170.59 100 - 1439	3.45 ± 1.55 0.15 - 20
10 normals	46.45 ± 6.32 20 - 93	22.62 ± 2.35 10 - 38	65,8 ± 3.91 21 - 84	0.50 ± 0.14 0.15 – 0.80
T-test	P < 0.0001	P = 0.001	P = 0.004	P = 0.023
Spearman's coefficient R	NMNp/TH : 0.51 NMNp/CgA : 0.71	MNp/TH : 0.81 MNp/CgA : 0.38	CgA/TH : 0.41	



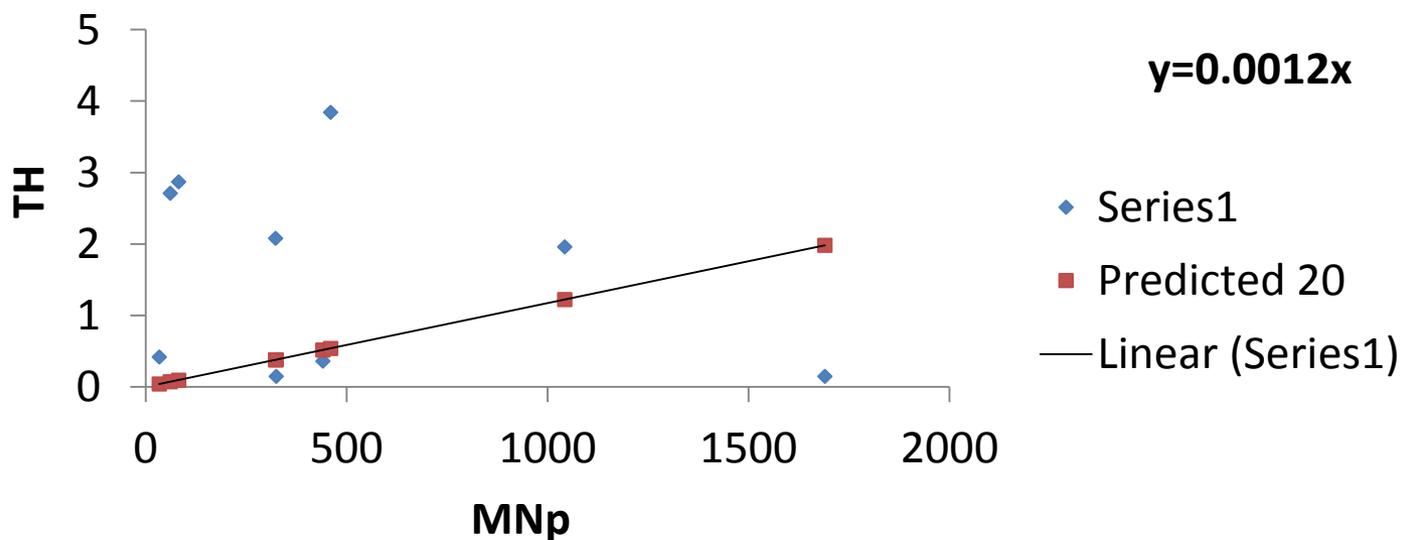
# Results and discussion

Fig.1 -Graphic correlation between plasma NMNp and TH



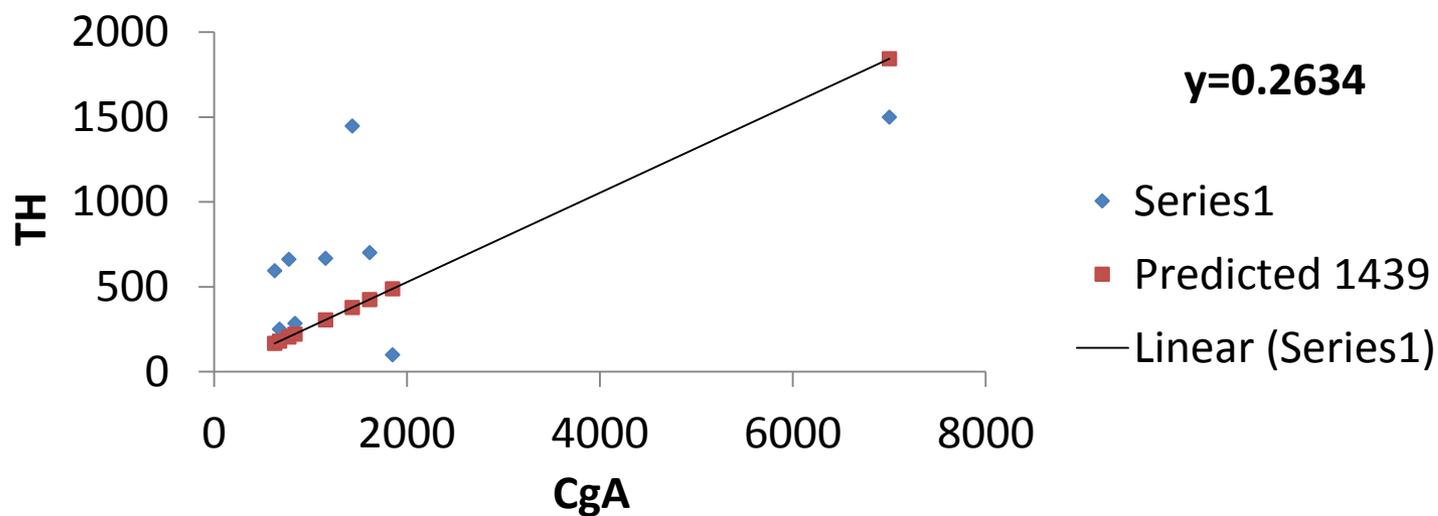
# Results and discussion

Fig.2 –Graphic correlation between plasma MNp and TH



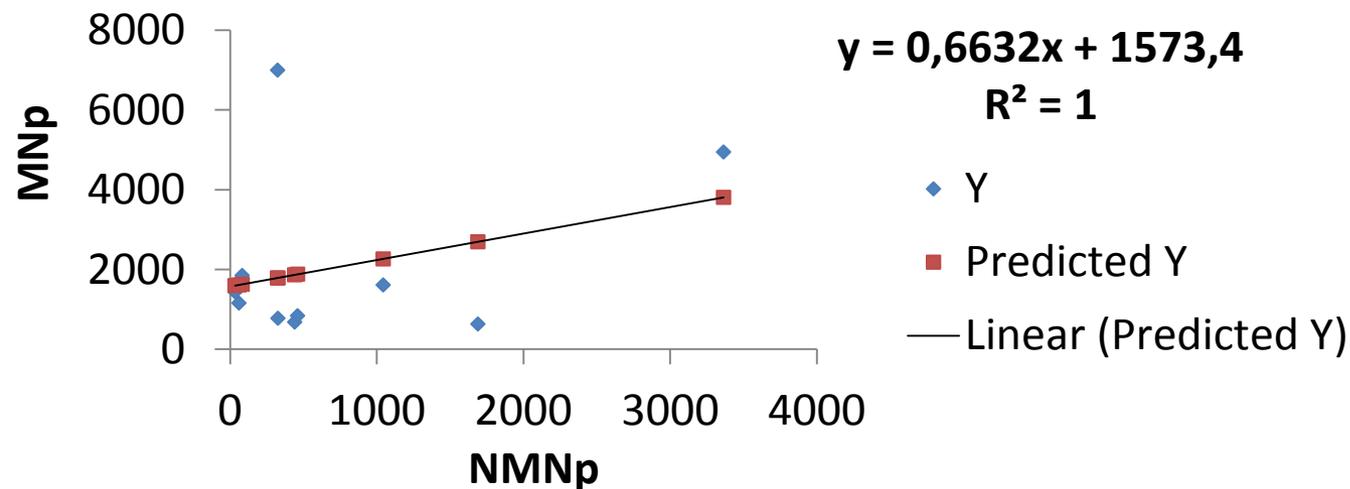
# Results and discussion

Fig.3- Graphic correlation between plasma CgA and TH



# Results and discussion

Fig.4- Graphic correlation between NMNp and MNp



# Conclusions

- Excess of catecholamines metabolites is well correlated with TH values proving a great rate of catecholamine biosynthesis and a large contribution of intratumoral catecholamines metabolism to elevated levels of plasma free normetanephrines/metanephrines in some cases of pheochromocytoma
- CgA colocalised with catecholamines in dense - core chromaffine granules is important in its relation with TH activity
- We could assert TH could be used as an index of functionality in pheochromocytoma diagnosis because it was well correlated with normetanephrines and especially with metanephrines content in our explored cases
- This study could be continued, exploring the so called “silent” pheochromocytomas having in mind biochemical, histochemical and genetical means



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