SHORT COMMUNICATION

PLASMA CATECHOLAMINE AND DOPAMINE β-HYDROXYLASE AMOUNTS IN PHAEOCHROMOCYTOMA

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SUMMARY

1. Plasma catecholamine and dopamine β-hydroxylase (DβH) amounts were measured in a series of patients with proven phaeochromocytoma and compared with those of a group with essential hypertension.

2. Circulating catecholamine amounts were greatly elevated in phaeochromocytoma but plasma DβH was not significantly raised.

3. There was a significant correlation between plasma noradrenaline and DβH in individuals with essential hypertension but not with phaeochromocytoma.

4. The relative deficiency of DβH secretion in phaeochromocytoma indicates that the mechanism of release of catecholamines from these tumours does not involve the normal exocytosis of catecholamine storage vesicles. This supports the hypothesis that the primary defect responsible for the hypertension is excessive synthesis of catecholamines that bypass the normal storage and secretion mechanisms and diffuse into the circulation.

Key words: plasma catecholamines, dopamine β-hydroxylase, phaeochromocytoma.

Phaeochromocytomas synthesize and secrete catecholamines at a higher rate than the normal adrenal medulla, but the mechanism for this increase is not known (Winkler & Smith, 1972). Since these tumours are not innervated, there are at least two possible explanations for the high rate of catecholamine synthesis and secretion. Either catecholamine storage is defective or the normal process of release of the hormones is disturbed. Winkler & Smith (1968) summarized the available evidence that storage of catecholamines in chromaffin vesicles was normal in phaeochromocytoma cells, but that catecholamine synthesis was excessive. They proposed that the primary defect leading to abnormal release of catecholamines was an uncoupling of the normal feedback mechanisms regulating synthesis rate in accordance with the amount of catecholamine stores. Hence newly synthesized catecholamines would bypass the saturated vesicle stores and diffuse into the circulation from the tumour.

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Secretion of catecholamines from the normal adrenal medulla and sympathetic nerves is now known to occur by exocytosis of the contents of catecholamine storage vesicles (Geffen & Livett, 1971; Smith, 1971). These vesicles contain and also release the enzyme dopamine β-hydroxylase (DβH), that forms noradrenaline from dopamine. The development of radioenzymic (Engelman & Portnoy, 1970) and radioimmunoassays (Rush & Geffen, 1972) for plasma catecholamines and DβH respectively has provided an opportunity to test one prediction of the hypothesis that secretion does not occur by exocytosis; namely, that the ratio of circulating DβH to catecholamines will be lowered in phaeochromocytoma compared with other forms of hypertension (Winkler & Smith, 1968).

Fig. 1. Individual values for circulating plasma catecholamines and dopamine β-hydroxylase in patients with phaeochromocytoma or essential hypertension.

MATERIALS AND METHODS

Assays

Plasma catecholamines (CA). These were measured by a double-isotope derivative method (Louis & Doyle, 1971). This method involves a preliminary separation of catecholamines by absorption on alumina, methylation using a [14C]methyl donor and conversion of the 14C-labelled normetanephrine into 14C-labelled vanillin. Purified [3H]noradrenaline is used as a marker for recovery. The sensitivity of the method allows accurate measurement of plasma samples containing 0.5 ng of CA.

Plasma DβH. This was measured by solid-state radioimmunoassay (Rush & Geffen, 1972). DβH was purified from sheep adrenals and antibodies to DβH were raised in rabbits. Purified DβH was iodinated with 125I and incubated in antibody-coated tubes with either standard amounts of unlabelled purified DβH or serum samples. After rinsing with water, the dry tubes were counted in a well counter (Nuclear–Chicago, model 8703). The DβH concentration of the serum was determined from the standard curve of the degree of inhibition of tracer binding. The assay was sensitive to 1 ng of sheep DβH and could detect DβH in 10 μl of
human serum. Since the degree of cross-immunoreactivity between species has not yet been
determined, results are expressed as the uncorrected sheep equivalents.

Patients

Eleven patients with phaeochromocytoma were studied. Plasma catecholamine amounts
were measured in all and in eight of these patients plasma DβH amounts were also measured.
The diagnosis of phaeochromocytoma was confirmed in all cases at operation, and by histo-
logical and biochemical analyses of the tumours.

Patients with essential hypertension were also studied. They were free of overt renal disease
and had never received antihypertensive drugs. All were admitted to hospital and blood was
drawn after 3 days bed rest at 09.00 hours on the third day after admission. Plasma catecho-
lamine amounts were measured in thirty-one patients and plasma DβH in twenty-one. Consent
was obtained from all the patients.

RESULTS

The individual values for plasma catecholamines and DβH in the two groups of patients are
shown in Fig. 1. Plasma amounts of catecholamines, but not DβH, were sharply elevated in
phaeochromocytoma compared with essential hypertension. The mean catecholamine concen-
tration in patients with phaeochromocytoma was 5.75±1.39 (SEM) ng/ml and in patients with
essential hypertension was 0.34±0.04 ng/ml (P<0.001). There was no significant difference in
plasma DβH amounts, the mean amounts being 329±81 and 226±29 ng/ml respectively
(P>0.10). Accordingly, the ratio DβH/catecholamines in peripheral blood was significantly
lower (P<0.001) in phaeochromocytoma (95±28) than in essential hypertension (661±84).
There was a significant positive correlation between plasma catecholamines and DβH amounts
in patients with essential hypertension (r = 0.61, P<0.01), but not with phaeochromocytoma
(r = 0.24).

In three patients with phaeochromocytoma studied post-operatively, both catecholamines
and DβH decreased after removal of the tumour. However, catecholamine amounts fell con-
siderably more than those of DβH, so that the mean DβH/catecholamine ratio in these patients
rose from 79 to 335. Thus while the tumours were secreting predominantly catecholamines, a
small amount of DβH was also being released.

DISCUSSION

The use of a sensitive specific radioimmunoassay (Rush & Geffen, 1972) for the measurement
of plasma DβH represents an advance over previous available enzymic methods (Weinshilboum
&Axelrod, 1971; Goldstein, Friedman & Bonnay, 1971), because radioimmunoassay measures
the total enzyme protein released rather than the highly variable residual activity of the enzyme
in blood. The significant correlation between circulating amounts of catecholamines and DβH
in individual patients without tumours strengthens the physiological evidence that the two
substances are released together from adrenergic nerve terminals.

In essential hypertension, combined estimations of plasma catecholamines and DβH
amounts may provide a useful measure of the contribution of the sympathetic nervous system
to the maintenance of the raised blood pressure. In phaeochromocytoma, however, the secre-
tion of catecholamines and DβH appears to be dissociated. The high circulating catecholamine amounts were not matched by a corresponding increase in plasma DβH, so that the mean ratio DβH/catecholamines was one-sixth of that in essential hypertension. It is unlikely that the tumours were secreting vesicle-protein fragments that were less immunoreactive since the amounts and electrophoretic pattern of soluble proteins in vesicles isolated from phaeochromocytomas are very similar to normal adrenal chromaffin vesicles (Winkler & Smith, 1972). The relative deficiency in circulating DβH pre-operatively, and the small fall post-operatively, compared with circulating catecholamines indicates that the tumours were not releasing catecholamines primarily from their storage vesicles. Rather, our results support the hypothesis that the basic defect in the tumour is an excessive synthesis of catecholamines that bypass the saturated storage sites and diffuse directly to the circulation (Winkler & Smith, 1968).

As Winkler & Smith (1972) have pointed out, an abnormal mechanism of release, involving diffusion of continuously synthesized catecholamines rather than neurally induced exocytosis of chromaffin vesicle stores, is compatible with the lack of innervation of these tumours, their high catecholamine turnover and metabolite production, and the rapidity with which α-methyl-tyrosine, an inhibitor of catecholamine synthesis, controls blood pressure before it has any effect on tumour catecholamine stores. Variations in blood flow and hence washout of catecholamines accumulated in the rich vascular spaces of the tumour, induced mechanically or by reflexes, could thus explain many of the well-known clinical fluctuations, as well as the provocative actions of vasodilator agents such as histamine and glucagon in doses that do not release catecholamines from the adrenal medulla.

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REFERENCES


