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Monoamine Oxidase and Catechol-O-Methyltransferase Activity in Hamster and Rat Insulinomas

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Summary. Hamster and rat insulinomas were assayed for norepinephrine, dopamine and serotonin concentration and for monoamine oxidase and catechol-o-ethyltransferase (COMT) activity. The concentration of norepinephrine (mean 0.55 μmol/kg, range <0.20 to 2.64 μmol/kg) and serotonin (mean 5.22 μmol/kg, range <0.6 to 26.5 μmol/kg) in hamster insulinomas were comparable to previously reported concentrations. Dopamine concentration (mean 0.34 μmol/kg, range <0.20 to 0.95 μmol/kg) was only 2 to 2.5% of that reported previously. Monoamine oxidase activity of the hamster and rat insulinomas were comparable to those of normal hamster islets. In contrast, the COMT activity of both insulinomas was much greater than the COMT activity of normal pancreatic islets of both species and was greater than in several other tissues and tumours. The tumour COMT, which was predominantly in the cytosol, was Mg^{2+} dependent and had a comparable sensitivity to inhibition by tropolone as purified beef-liver COMT. Hamster insulinoma monoamine oxidase was more sensitive than rat insulinoma monoamine oxidase to inhibition by tranylcypromine and deprenyl, while rat insulinoma monoamine oxidase was more sensitive to inhibition by clorgyline and was more heat labile.

Key words: Insulinoma, monoamine oxidase, catechol-o-ethyltransferase, hypoglycaemia, norepinephrine, dopamine, serotonin, neoplasms, tropolone, tranylcypromine, clorgyline, deprenyl.

Considerable evidence has accumulated to demonstrate that monoamines such as epinephrine [1, 2], norepinephrine (NE) [3], dopamine [4] and serotonin [4] are potent inhibitors of insulin secretion from normal pancreatic islets. Histochemical fluorescence data indicates that the pancreatic islets of some, but not all, species contains nonoamines [5]. For example, golden hamster islets have a rich adrenergic nerve plexus and these nerves contain a large amount of NE; rat islets have only sparse adrenergic nerves with only trace amounts of NE [5]. Neither the hamster or rat has stainable monoamines in the B or A cells of the islets [5].

Some human insulinomas are reported to contain substantial amounts of dopamine and serotonin [6–8]; monoamines have not been identified in other human insulinomas [9]. A transplantable islet cell tumour of the golden hamster (hamster) contains substantial amounts of dopamine, serotonin, L-3,4 dihydroxyphenylalanine (L-DOPA) and an unidentified monoamine-like substance [10–13]. Although the hamster islet cell tumour synthesizes and secretes insulin, its insulin content is low [14]. Chick et al. recently described a transplantable rat insulinoma that contains and secretes large amounts of insulin [15]. This tumour has not been evaluated for its monoamine content.

It is paradoxical that islet cell tumours that contain substances that inhibit insulin secretion from normal islets (dopamine, serotonin) are characterized by brisk insulin secretion. The present study compares the monoamine content of the newly-characterized rat insulinoma with the monoamine content of the hamster insulinoma. In addition, the activity of the 2 principle enzymes inactivating monoamines, monoamine oxidase (monoamine: oxygen oxidoreductase, EC 1.4.3.4.) and catechol-o-methyltransferase (S-adenosyl-L-methionine: catechol-o-methyltransferase, EC 2.1.1.6.), were assessed in hamster and rat insulinomas. Monoamine oxidase (MAO) is present in hamster and rat islets and catechol-o-methyltransferase (COMT) is present in hamster islets [16, 17].

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Table 1. Monoamine oxidase and COMT activity and monoamine concentration in insulinomas

	Hamster	Rat
MAO activity (pmol product/mg protein/min)	2331±158[a]	897±103[a]
COMT activity (pmol product/mg protein/min)	314± 24	327± 49
Norepinephrine concentration (μmol/kg)	0.55 (<0.20–2.64)	<0.20
Dopamine concentration (μmol/kg)	0.34 (<0.20–0.95)	<0.20
Serotonin concentration (μmol/kg)	5.22 (<0.6–26.5)	3.58 (<0.6–10.9)

Results given as mean ± SEM or mean (range) of 10 hamster and 6 rat insulinomas

[a] Significantly different with $p<0.01$

Materials and Methods

Materials

The following materials were purchased from commercial sources: collagenase, class IV (Worthington Biochemical Co., Freehold, NJ), tryptamine bisuccinate (side chain -2-^{14}C) with specific activity of 60 mCi/mmol and S-adenosyl-L-(^{3}H-methyl)-methionine (^{3}H-SAM) with specific activity of 7.5 Ci/mmol (New England Nuclear, Boston, MA); freeze-dried beef-liver COMT (Miles Laboratory, Elkhart, IN); tropolone (K&K Fine Chemicals, Plainview, NY); and male golden hamsters (100–125 g) (Engel Laboratory Inc., Farmersburg, IN).

The following materials were gifts: tranylcypromine sulphate (Smith, Kline & French Laboratory, Philadelphia, PA); clorgyline HCl (Dr. K. Gaimster, May & Baker, Ltd., Dagenham, England); deprenyl HCl (Dr. J. Knoll, Semmelweis University of Medicine, Budapest, Hungary); hamster insulinoma (Dr. S. Shin, Albert Einstein College of Medicine, Bronx, NY); rat insulinoma and male NEDH rats (Dr. William Chick and Dr. Eugene Knox, Boston, MA); and rat pituitary tumour GH-1, F-1001, CCL82 (American Type Culture Collections, Rockville, MD).

Experimental Procedure

The hamster insulinoma was minced and transplanted into the subcutaneous tissues of the hamster with a number 18 gauge needle. At approximately 3 weeks the hamster tumour host developed hypoglycaemia (plasma glucose less than 30 mg/dl) with plasma insulin 2 to 3 times greater than normal. The rat insulinoma was cut into small fragments and surgically implanted into the subcutaneous tissue of NEDH white rats. At approximately 4 weeks the rat tumour host developed hypoglycaemia with increased plasma insulin. Blood samples were obtained for glucose and insulin assay from the orbital sinus. When the tumour was ready for harvesting, the animals were either lightly anesthetized with ether or decapitated and the tumour removed. The tumour was stored in the freezer (−30 °C) until analyzed for enzyme activity, monoamine concent, and, in some cases, insulin content. When evaluating tissue MAO activity or monoamine content, the animals were sacrificed by decapitation and the appropriate tissues were removed. Pancreatic islets were isolated by a modified collagenase digestion technique [18]. The tissue was digested in Hanks balanced salt solution (pH = 7.4) when islet NE and MAO were determined [19]. When COMT was determined, calcium ion was omitted from the final wash as calcium is reported to inhibit COMT [20]. The tissues for NE and dopamine analysis were homogenized in 0.1 mol/l perchloric acid and the tissues for serotonin were homogenized in 0.1 mol/l HCl (3 °C in a ground-glass homogenizer). After the homogenate was centrifuged at 4000 X g, the supernatant was analysed for NE, dopamine or serotonin. The tissues for assay for MAO and COMT were homogenised in potassium chloride. Enzyme assays were done on total homogenate unless otherwise specified.

To prepare mitochondria-rich pellets and cytosol fractions, insulinomas were homogenized with a glass pestle in pH 7.8 buffer (0.25 mol/l sucrose, 0.15 mol/l KCl and 0.01 mol/l potassium phosphate). The homogenate was centrifuged in a Sorvall refrigerated centrifuge (4 °C) at 90 X g to remove nuclei, cell membranes, and unbroken cells. The supernatant fluid was then centrifuged at 4 °C at 27,700 X g for 15 min and the cytosol-containing supernatant fluid was decanted from the mitochondria-rich pellet.

In the studies with the COMT inhibitor tropolone, the tropolone was not preincubated with the enzyme. In the MAO inhibitor experiments, the inhibitor under investigation was dissolved in 0.5 mol/l sodium phosphate buffer (pH = 7.4). The inhibitor was preincubated with enzyme at room temperature for 5 min prior to addition of substrate. Tryptamine substrate was then added and the tubes incubated at 37 °C. MAO activity of the aliquots that were exposed to various concentrations of inhibitor were expressed as % activity of the control aliquots.

In the heat-inactivation study, tissue homogenates were divided into aliquots and suspended in 0.5 mol/l sodium phosphate buffer (pH = 7.4). Aliquots were incubated at room temperature or at 50 °C. Aliquots were removed after 2.5, 5, 10, 15 or 20 min and rapidly cooled at 0 °C. After return to room temperature, aliquots were simultaneously assayed for MAO activity. The MAO activity of the heated aliquots was expressed as % activity of the unheated aliquots.

Analytical Methods

MAO activity of tissue homogenates was determined with a sensitive radioassay that employed ^{14}C-tryptamine as a substrate [21]. MAO activity was equal to the amount of ^{14}-C-labelled indoleacetaldehyde and indoleacetic acid formed. These metabolites were isolated by differential solvent extraction and counted in a liquid scintillation counter. COMT activity was determined by a radioassay method in which COMT transfers a ^{14}C-S-adenosyl-L-methionine to 3,4-dihydroxybenzoic acid [22]. The ^{14}-C-O-methylated benzoate derivative was isolated by differential solvent extraction and counted in a liquid scintillation counter. All measurements were performed in duplicate with 2 different sized aliquots of homogenate. Substrate concentrations and incubation conditions were selected to give maximal activity. The protein content of the homogenates was determined colorimetrically [23]. The specific activity of the MAO and COMT activity is expressed as pmol/product formed/mg tissue protein/min incubation.

The radioenzymatic method for measuring NE utilised the enzyme phenyl-ethanolamine-N-methyltransferase (PNMT) that was partially purified from fresh bovine adrenal glands [24]. The PNMT transfers a ^{3}H-labelled methyl group from ^{3}H-SAM to NE in the tumour extracts. Due to differences in the nonradioactive SAM content of different tissues, each sample was quantitated with its own internal NE standard. There was less than 2% cross-reactivity with epinephrine, L-DOPA, dopamine or serotonin.

The radioenzymatic method for measuring dopamine utilises the enzyme COMT partially purified from beef liver [25]. The

Table 2. Comparison of monoamine oxidase and COMT activity in insulinomas and in other tissues

	Hamster		Rat	
	MAO[a]	COMT	MAO[a]	COMT
	(pmol product/mg protein/min)			
Insulinoma	2,331±158 1,900±190[b]	314±24	897±103 584± 95[b]	327± 49
Pancreatic islets	2,800±300[b]	7± 2	872± 54[b]	8± 3
Acinar pancreas	417± 74	62±13	1,020±130	46± 3
Cerebral cortex	716± 80	28± 2	1,636±292	54± 6
Median eminence	733±115	33± 6	3,000±900	87± 8
Anterior pituitary gland	460± 70	28± 5	826± 86	61± 12
Heart	143± 30	17± 1	460± 95	36± 2
Liver	1,850±250	74±16	2,948±528	1,963±234
Kidney	959± 86	714±62	486±100	842±115

Results given as mean ± SEM of 10 hamster and 6 rat insulinomas and normal tissues from 6 hamsters and 4 rats

[a] Unless otherwise noted, tryptamine concentration 1.0 mmol/l
[b] Tryptamine concentration 0.1 mmol/l

COMT transfers a ^{3}H-labelled methyl group from ^{3}H-SAM to the hydroxyl group in the 3 position of the benzene ring of the catecholamines in tissues. The (^{3}H-methyl)-methoxytyramine is then separated from the (^{3}H-methyl)-methoxy-beta-hydroxylated derivatives of epinephrine and NE by selective oxidation and differential solvent extraction. The oxidation step was carried out at room temperature and the reaction allowed to proceed for 4 min before stopping the oxidation by addition of glycerol. The dopamine content of each sample was quantitated with its own internal dopamine standard. Under the experimental conditions used, there was less than 1% cross-reactivity with L-DOPA, NE and serotonin, and 7% cross-reactivity with epinephrine.

The serotonin content of the insulinomas was determined fluorometrically [26] and the Mg^{2+} content colorimetrically [27]. Plasma glucose was determined with a glucose oxidase method, and plasma and tumour insulin by a double-antibody radioimmunoassay [28, 29]. The insulin was extracted from the insulinomas by acid-ethanol [30]. The extract was diluted with 0.01 mmol/l sodium phosphate buffer (pH = 7.4).

Statistical Methods

Unless otherwise stated, data are given as means ± SEM. The statistical significance of the differences between groups was sought with Student's t test.

Results

Table 1 compares the MAO and COMT activity in the hamster insulinoma and the rat insulinoma. Although the MAO activity of both insulinomas was high, the MAO activity of the hamster insulinoma was significantly greater than the MAO activity of the rat insulinoma ($p<0.01$). The COMT activity of both insulinomas was high and similar in the 2 tumours.

Although there was a wide range of values, the NE concentration was measurable in 8 of the 10 hamster insulinomas and the dopamine concentration was measurable in 7 hamster insulinomas. The concentration of NE and dopamine was below the level of sensitivity in all of the samples of rat insulinoma that were examined. The mean serotonin concentration in the hamster insulinoma was at least 10 times greater than the NE and dopamine concentration in this tumour. There was measurable serotonin in 5 of the 6 rat insulinomas.

Table 2 compares the MAO and COMT activity in the insulinomas with MAO and COMT activity of other hamster and rat tissues. The MAO activity of rat and hamster insulinomas were comparable to the MAO activity of normal rat and hamster pancreatic islets respectively. These values in the hamster were

Table 3. Fractionation of monoamine oxidase and COMT activity in insulinomas

	Hamster		Rat	
	MAO	COMT	MAO	COMT
	(pmol product/mg protein/min)			
Fraction				
Tissue homogenate	3,368±326	332±89	855± 98	290±24
90 X g Supernatant	1,326±148	401±64	672± 72	300±70
27,700 X g Supernatant	292± 42	541±52	89± 24	438±30
27,700 X g Pellet	4,811±173	87± 6	2,062±243	52±17

Results given as mean ± SEM of 4 tumours

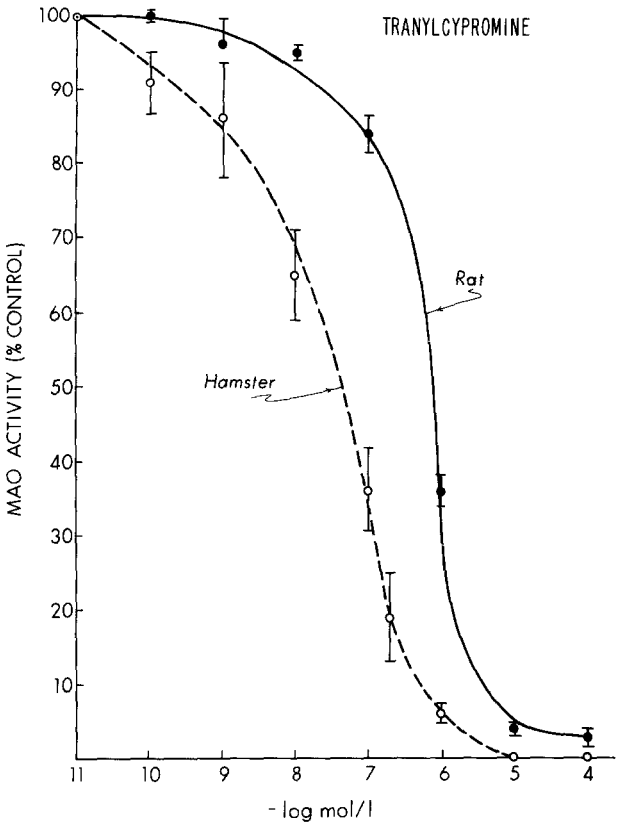

Fig. 1. Comparison of the effect of tranylcypromine on the MAO activity of homogenates of hamster and rat insulinomas. The inhibition of hamster tumour MAO by tranylcypromine was significantly greater than that in the rat at tranylcypromine concentrations ranging from 0.01 to 1.0 μmol/l ($p<0.01$). The tryptamine substrate concentration was 0.1 mmol/l. Each point represents the mean ± SEM of 5 to 8 different insulinomas

higher than in all other tissues studied with the exception of the liver. In contrast, with the exception of the heart and kidney, normal rat tissues all had MAO activity equal to or greater than that of the rat insulinoma or rat islets.

In contrast to the MAO activity, the COMT activity of the hamster and the rat insulinomas was much greater than the COMT activity of the normal islets (Table 2). The COMT activity of the hamster insulinoma was greater than the COMT activity of all of the hamster tissues analysed with the exception of the kidney. The COMT activity of the rat insulinoma was greater than the COMT activity of all the rat tissues analysed with the exception of the kidney and the liver.

Table 3 depicts the results of assaying subcellular fractions of hamster and rat insulinomas for MAO and COMT activity. The majority of the MAO activity of the hamster and rat insulinomas was in the 27,700 X g mitochondria-rich pellet with only a small amount of MAO activity in the 27,700 X g cytosol fraction. The majority of the COMT activity of the hamster and rat insulinomas is in the 27,700 X g cytosol fraction with only a small amount of COMT activity in the 27,700 X g mitochondria-rich pellet.

A series of studies were done to compare the MAO activity in the hamster and rat insulinomas. Figure 1 depicts experiments that demonstrate the MAO activity of the hamster insulinoma was significantly more sensitive to inhibition by the MAO inhibitor tranylcypromine than was that of the rat insulinoma. Figure 2 demonstrates that the MAO activity of the hamster insulinoma was more sensitive to inhibition by deprenyl, while that of the rat insulinoma was more sensitive to inhibition by clorgyline.

Figure 3 illustrates the alterations in MAO activity of hamster and rat insulinomas following exposure of the homogenate to a temperature of 50 °C. The MAO activity of the rat insulinoma was much more heat labile than that of the hamster insulinoma.

A series of studies were done to compare the COMT activity of hamster and rat insulinomas with the COMT activity of purified beef-liver COMT. COMT activity was assayed in the absence and presence of 3 mmol/l Mg^{2+} (Table 4). In Mg^{2+} free medium there was a 63%, 85% and 91% loss of COMT activity in hamster insulinoma, rat insulinoma and bovine liver COMT respectively. The Mg^{2+} content of hamster and rat insulinomas ranged from 2 to 4 mmol/kg wet weight with no Mg^{2+} detectable in the bovine liver COMT. When the COMT assay was carried out in the presence of 0.1 mmol/l ethylenediamine-tetraacetic acid (EDTA) there was a 97 to 100% loss of COMT activity in the insulinomas and purified bovine liver COMT. This was not due to a "toxic" effect of EDTA on COMT, for 3 preparations had almost full COMT activity when EDTA (0.1 mmol/l) was added to Mg^{2+} (3 mmol/l) containing media.

The effect of the COMT inhibitor tropolone on the methylating activity of 4 hamster insulinomas, 4 rat insulinomas and 4 batches of purified bovine-liver COMT was evaluated [32]. At 0.1 mmol/l, tropolone caused a 66 ± 6% (hamster insulinoma), 57 ± 5% (rat insulinoma) and 69 ± 5% (bovine-liver COMT) inhibition of methylating activity. At 1 mmol/l, tropolone caused a 95 ± 1% (hamster insulinoma), 93 ± 2% (rat insulinoma) and 95 ± 2% (bovine-liver COMT) inhibition of methylating activity.

To determine if the markedly elevated COMT, and to a lesser extent MAO, activity is common to neoplasms, the activity of these enzymes was assayed in a transplantable rat pituitary adenoma and in 6 human neoplasms (adenocarcinoma of the lung, ileal carcinoid, phaeochromocytoma, papillary thyroid

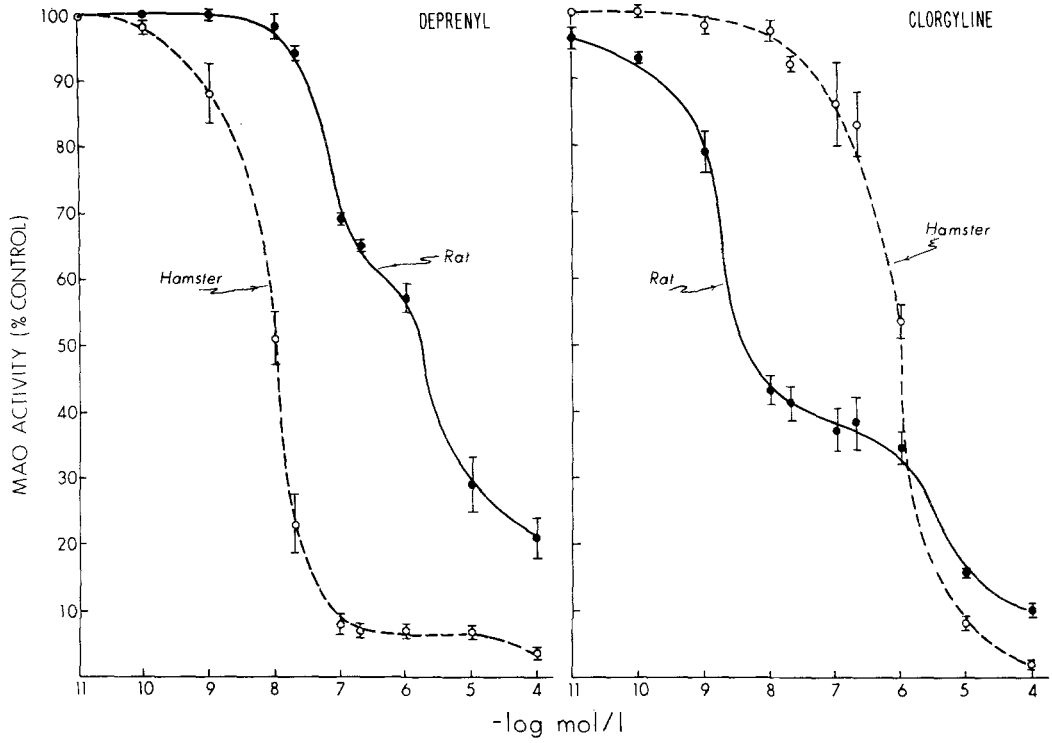

Fig. 2. Comparison of the effect of deprenyl and clorgyline on the MAO activity of homogenates of hamster and rat insulinomas. The inhibition of hamster tumour MAO by deprenyl was significantly greater than inhibition in the rat at deprenyl concentrations ranging from 1 nmol/l to 0.1 mmol/l. The inhibition of rat tumour MAO by clorgyline is significantly greater than in the hamster at clorgyline concentrations ranging from 1 nmol/l to 1 μmol/l ($p<0.01$). The tryptamine substrate concentration was 0.1 mmol/l. Each point represents the mean ± SEM of 5 to 8 different insulinomas

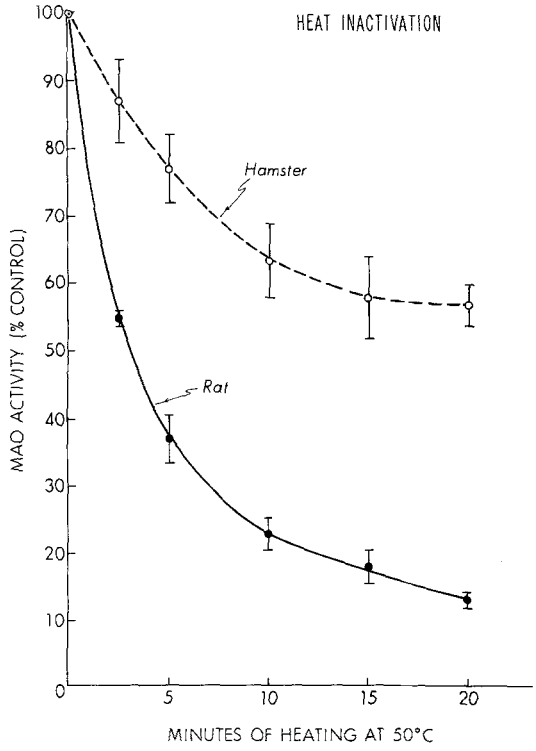

Table 4. Effect of Mg^{2+} on COMT activity of hamster and rat insulinoma and purified bovine liver COMT

	COMT activity (pmol product/mg protein/min)		
	Mg^{2+}[a] containing medium	Mg^{2+} free medium	Mg^{2+} medium with EDTA[b]
Hamster	361± 9	99±7 (63%)[c]	10±2 (97%)
Rat	336±47	49±12 (85%)	5±1 (99%)
Bovine liver COMT	1,381±64	115±8 (91%)	6±3 (100%)

Results are given as mean ± SEM of 4 insulinomas or 4 batches of purified bovine liver COMT

[a] Mg^{2+} concentration in medium 3 mmol/l
[b] EDTA concentration in medium 0.1 mmol/l
[c] Inhibition of COMT activity is in parentheses

Fig. 3. Comparison of the effect of heat on the stability of MAO activity of homogenates of hamster and rat insulinomas. The homogenates were heated to 50 °C for varying periods of time as described in Methods. Each point represents the mean ± SEM of 6 different insulinomas

carcinoma, small cell thyroid carcinoma and follicular thyroid adenoma). The COMT activity of the insulinomas was 3 times greater than the COMT activity of the rat pituitary adenoma and at least 60 times higher than any of the human neoplasms. The MAO activity of the insulinomas was greater than that of 5 of the 7 neoplasms.

Discussion

The present study indicates that the MAO activity of the hamster and rat insulinomas were similar to that of normal hamster and rat islets. This monoamine oxidising activity appears to be real MAO activity for it is predominantly associated with the mitochondrial pellet and it is extremely sensitive to inhibition by known MAO inhibitors [33–35].

In the past there have been only a limited number of studies on the MAO activity of neoplasms. Using serotonin as the substrate, Foa noted that the MAO activity of the hamster insulinoma was 3 times greater than that of the hamster pancreas [12]. Apparently he did not measure the MAO activity of normal pancreatic islets [12]. Using tryptamine as the MAO substrate, the present study indicates that the hamster insulinoma has 5.5 times the MAO activity of the pancreas. The hamster insulinoma and normal hamster islets have comparable MAO activity. Kizer and Chan noted that 5 of 7 mouse and rat hepatomas studied had no MAO activity [36].

MAO exists in several isoenzymes. The isoenzymes differ on the basis of their substrate specificity, inhibitor sensitivity, thermostability, antigenic properties and electrophoretic mobility [17]. The existence of 2 forms of MAO in rat brain, termed A&B, has been inferred from in vitro and in vivo experiments [37, 38]. Enzyme A is heat stable, sensitive to clorgyline and resistant to deprenyl and tranylcypromine [34, 35]. Enzyme B is heat labile, resistant to clorgyline and sensitive to deprenyl and tranylcypromine [34, 35].

Tryptamine, the MAO substrate used in the present study, is metabolised by both the A and B enzyme [34]. With tryptamine as the substrate, hamster insulinoma MAO was heat stable, sensitive to inhibition by tranylcypromine and deprenyl, and resistant to inhibition by clorgyline. The heat stability suggests it is an A type enzyme while the sensitivity to tranylcypromine and deprenyl suggests it is a B type enzyme. The rat insulinoma MAO was heat labile sensitive to inhibition by clorgyline and resistant to inhibition by tranylcypromine and deprenyl. The heat lability suggests it is a B type enzyme while the sensitivity to clorgyline suggests it is an A type enzyme. Thus, one cannot classify hamster and rat insulinoma MAO as to A or B type by these criteria.

In contrast to the findings with MAO, the COMT activity of both the hamster and rat insulinomas was much greater than the COMT activity of hamster or rat pancreatic islets. The COMT activity of the insulinomas was greater than the COMT activity of the majority of normal hamster and rat tissues. Increased COMT activity is not a general property of neoplasms for the COMT activity of insulinomas was greater than the COMT activity of 7 miscellaneous neoplasms that were evaluated.

There are a number of other methylating enzymes that utilise SAM as a cofactor. These include phenethanolamine-N-methyltransferase, nonspecific phenylethylamine-N-methyltransferase, histamine-N-methyltransferase and hydroxyindole-O-methyltransferase. Although these enzymes are predominantly located in the cytosol, they are not Mg^{2+} dependent or inhibited by tropolone [39]. The methylating activity we noted in the insulinomas with the 3,4-dihydroxybenzoic acid substrate was predominantly found in the cytosol, Mg^{2+} dependent and sensitive to inhibition by tropolone. It thus appears to be authentic COMT activity [39].

We noted that the hamster insulinoma contains more NE and dopamine than the rat insulinoma. Histochemical fluorescence studies indicate that normal hamster islets contain more NE than normal rat islets [5]. The NE and serotonin content of the hamster insulinoma was comparable to that previously reported by other investigators [11–13]. However, the mean concentration of dopamine (0.34 μmoles/kg) we found in the hamster insulinoma was only 2.0 to 2.5% of that reported by previous investigators [11–13].

This difference in dopamine content may be due to differences in the characteristics of the tumour that have developed over the years. However, the low insulin content of the tumour that we noted (65 ± 17 mU/g) was similar to the insulin content of the tumour that was previously described (72 ± 14 mU/g) [14]. This suggests that at least some of the characteristics of the tumour have been relatively stable over the years.

Another possibility for the difference in dopamine content of the hamster insulinoma is methodological. We utilised a highly specific radioenzymatic assay for dopamine while previous investigators utilized fluorescent techniques [11–13]. Cegrell et al. noted that the hamster insulinoma contained an unknown substance whose formaldehyde-induced fluorophore dominated the recordings of the fluorescence spectra [13]. This identified substance

may interfere with the fluorescent method of measurement of dopamine analysis.

Unpublished observations from our laboratory indicate that normal hamster islets have a 10 to 70 fold greater concentration of NE, dopamine and serotonin than the hamster insulinoma. One might speculate that the NE and dopamine in the insulinoma is lower than the NE and dopamine of pancreatic islets because of the increased COMT activity in the insulinoma. However, COMT does not metabolise serotonin.

Recent studies indicate that many of the factors that modify insulin secretion from normal islets also modify insulin secretion from the hamster insulinoma [40]. Studies of the possible role of monoamines and monoamine degrading enzymes (MAO, COMT) in the regulation of insulin secretion from insulinomas should be of interest.

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