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Characteristics of Procarbazine as an Inhibitor In-vitro of Rat Semicarbazide-sensitive Amine Oxidase

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Abstract—Procarbazine (*N*-isopropyl-α-(2-methyl hydrazino)-*p*-toluamide hydrochloride) inhibited more powerfully the deamination of benzylamine by semicarbazide-sensitive amine oxidase (SSAO) of rat brown adipose tissue than the deamination of 5-hydroxytryptamine and benzylamine by rat liver monoamine oxidase-A or -B activities, respectively. Inhibition of SSAO, but not monoamine oxidase, was time-dependent. Use of metabolic inhibitors, and an enzyme dilution technique, suggested that any conversion of procarbazine to an active species must be as a result of the action of SSAO itself and not of any other enzyme. The non-competitive kinetics and the time-dependence of inhibition were indicative of a suicide interaction between procarbazine and SSAO. The slow reversal of inhibition by dialysis was evidence in favour of the involvement of tight binding, rather than covalent bonding. High concentrations of benzylamine afforded the enzyme significant protection from the action of procarbazine, indicating that the interaction is at or near the active site. If the properties of procarbazine, evident in in-vitro studies, are retained in-vivo, these data suggest that procarbazine might be suitable for the examination of SSAO activities, both in-vivo and ex-vivo.

Amine oxidase activities, resistant in-vitro to the acetylenic inhibitors of monoamine oxidase (MAO; EC 1.4.3.4), but which are inhibited by carbonyl compounds such as semicarbazide, have been classified as EC 1.4.3.6 and are known collectively as semicarbazide-sensitive amine oxidase (SSAO) enzymes. Existing in both tissue-bound and soluble forms, these SSAO enzymes have, like MAO, a wide distribution throughout the animal kingdom and are also associated with plants and microorganisms (see Callingham & Barrand (1987) for review). However, while their molecular and mechanistic aspects continue to be examined in some detail (Bruinenberg et al 1989; Janes & Klinman 1991; Hartmann & Klinman 1991), a physiological role for most of these enzymes has yet to be found. This can be attributed, in the main, to a lack of highly selective inhibitors. Such compounds would allow the modification of SSAO activity without affecting MAO and other enzymes sensitive to the current range of inhibitors of SSAO. This would be advantageous particularly in in-vivo studies and in experiments using isolated organs and tissues where changes in pharmacological responses and metabolite patterns could be compared.

The carcinostatic agent, procarbazine (*N*-isopropyl-α-(2-methyl hydrazino)-*p*-toluamide hydrochloride) has been shown to inhibit benzylamine oxidation by SSAO in homogenates of various human tissues and of rat lung (Lewinsohn et al 1978). In an attempt to find a potent, irreversible SSAO inhibitor, which could be used in-vivo, we have examined in greater detail the effects in-vitro of procarbazine on rat amine oxidase activities, using brown adipose tissue (brown fat) and liver as sources of SSAO and MAO, respectively (Barrand & Callingham 1982).

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Materials and Methods

Materials

Substrates for the radiochemical amine oxidase assays were [7-^{14}C]benzylamine hydrochloride, from ICN Flow (High Wycombe, Bucks, UK) and 5-hydroxy[G-^{3}H]tryptamine creatinine sulphate, from Amersham International (Amersham, Bucks, UK). Unlabelled benzylamine was obtained as the free base from Sigma Chemical Co. (Poole, Dorset, UK) and crystallized as the hydrochloride in the laboratory. Unlabelled 5-hydroxytryptamine creatinine sulphate was obtained from Sigma. Opti-fluor O (liquid scintillation fluid) was obtained from Canberra Packard (Pangbourne, Berks, UK).

Clorgyline hydrochloride was a gift from Rhône-Poulenc (Eccles, Manchester, UK). Pargyline hydrochloride was purchased from Sigma and proadifen hydrochloride (SKF 525-A) was a gift from Smith Kline and French Laboratories (Welwyn Garden City, Herts, UK, now SmithKline Beecham, Harlow, Essex, UK). Procarbazine, a gift from Hoffmann-La Roche (Basel, Switzerland), was obtained as the free base and dissolved with an equivalent amount of HCl in aqueous solution. All other reagents were of analytical grade where possible. Male Wistar rats, 200–400 g, were supplied by A. J. Tuck and Son (Rayleigh, Essex, UK).

Preparation of homogenates

Rats were killed by stunning followed by decapitation. Livers were excised, washed and stored at −20°C until required. Pads of interscapular brown fat were removed and placed on ice. A pooled homogenate of brown fat, obtained from a minimum of 3 animals, was made (1 in 40 w/v in 0·2 M potassium phosphate buffer, pH 7·8) using a mechanical homogenizer (Polytron Mark 5, Kinematica). The homogenate was stored in 1 mL portions at −70°C until required.

Liver homogenates were made immediately before use (1 in 40 w/v in 1 mM potassium phosphate buffer, pH 7·8), using a Polytron homogenizer.

Radiochemical amine oxidase assay

Amine oxidase assays were based on the method described by Lyles & Callingham (1982). Assays, carried out in triplicate, were set up in ice-cooled disposable glass tubes containing 25 μL of homogenate, 25 μL of distilled water or inhibitor in aqueous solution and 50 μL of appropriate radiolabelled substrate. Samples were usually oxygenated and pre-incubated with inhibitors at 37°C in stoppered tubes before addition of substrate. Following addition of substrate, samples were re-oxygenated and incubated for 10 min (5 min in kinetic experiments) at 37°C. Enzyme activity was terminated by plunging the tubes into ice and adding HCl (3 M, 10 μL) to each. Blanks had HCl added before incubation with substrate. Deaminated metabolites were extracted into 0·7 mL ethyl acetate/toluene (1:1 v/v, saturated with water) and 0·4 mL of the organic phase was counted in 4 mL of Opti-fluor O for radioactive metabolites, with quench correction by external standardization in a scintillation spectrometer (Packard model 2500 TR). Substrates used were, for SSAO, [^{14}C]benzylamine (10 μM, sp. act. 10 μCi μmol^{-1}), for MAO-A, [^{3}H]5-hydroxytryptamine (250 μM, sp. act. 2 μCi μmol^{-1}) and for MAO-B, [^{14}C]benzylamine (250 μM, sp. act. 2 μCi μmol^{-1}).

Inhibitor selectivity and potency

In experiments to determine the selectivity of procarbazine for SSAO over MAO, samples were pre-incubated at 37°C for 3 h with procarbazine or distilled water (controls) before remaining enzyme activity was assayed by addition of labelled substrate, as described above. Preliminary experiments indicated that, at submaximal concentrations of procarbazine, a pre-incubation period of between 2 and 3 h was necessary for the amount of inhibition to reach a plateau.

Determination of the involvement of metabolic enzymes

Both MAO and cytochrome P450 are thought to be involved in the metabolism of procarbazine in-vivo (Schwartz 1966; Coomes & Prough 1983). To determine whether or not these enzymes are involved in the conversion of procarbazine to an inhibitor of SSAO, brown fat homogenate (1:40) was first pre-incubated with clorgyline (500 μM) or proadifen (500 μM), under oxygen in stoppered tubes at 37°C for 30 min, to inhibit completely MAO and cytochrome P450 activities, respectively. These inhibitors were not added to controls or to samples designed to show the effect of procarbazine alone. Samples were then pre-incubated with procarbazine (1 μM) or distilled water (controls) at 37°C for 1 h and remaining SSAO activity was assayed as described above.

To determine whether or not any other activating enzyme was present, the effect of diluting the homogenate was examined. Two dilutions of brown fat homogenate (1:40, 400 μL and 1:200, 400 μL) were pre-incubated with 400 μL of an aqueous mixture of clorgyline and proadifen (both at 500 μM), at 37°C for 30 min under oxygen. Samples were then divided into two portions of 400 μL and pre-incubated further with distilled water (400 μL) or procarbazine (4 μM, 400 μL) at 37°C for 1 h under oxygen. Samples (50 μL) were assayed for remaining SSAO activity by addition of labelled substrate, as described above.

Dialysis experiments

For dialysis studies, 3 mL of brown fat homogenate (1:40) was pre-incubated with 3 mL of distilled water or 10 μM procarbazine at 37°C for 2 h and then 0·75 mL aliquots of the resulting samples were dialysed under appropriate conditions (see Results) for 6 or 12 h. Non-dialysed samples were also stored at dialysis temperatures. SSAO activity in each sample was then assayed as described above.

Protection of the active site

The active site of SSAO was protected by pre-incubating homogenate and procarbazine in the presence of unlabelled benzylamine. Brown fat (1:40, 500 μL) was pre-incubated under oxygen at 37°C for 1 h, with distilled water (500 μL), benzylamine (100 μM, 500 μL), procarbazine (2 μM, 500 μL) or 500 μL of an aqueous solution containing procarbazine (2 μM) and benzylamine (100 μM). Tubes were placed on ice, 4 mL of 1 mM potassium phosphate buffer (pH 7·8) was added and the samples centrifuged at 1500 *g* for 15 min (MSE Chilspin). The supernatants were discarded and the pellets resuspended in 5 mL of buffer. Centrifugation and resuspension were repeated twice and the pellets were finally resuspended in 600 μL of buffer. Remaining SSAO activity was assayed in 50 μL aliquots of each sample as described above.

Protein assays

Protein contents of homogenates were measured by the method of Lowry et al (1951) and enzyme activities are expressed as nmol of product formed h^{-1} (mg protein)$^{-1}$.

Statistical analysis

Values are expressed as the mean $\pm$ s.e.m. Sigmoid curves were fitted to the data, when appropriate, using the nonlinear regression facility of GraphPAD InPlot, Version 3·0 (GraphPAD Software, San Diego, CA, USA).

Results

Potency and selectivity of procarbazine against some amine oxidase activities

Homogenates of brown fat or liver were pre-incubated for 3 h with procarbazine concentrations from 0·1 nM to 0·1 M and remaining amine oxidase activities were determined. Fig. 1 illustrates the concentration-dependent inhibition of enzyme activities compared with activity in control samples. The IC50 values obtained following fitting of sigmoid curves to the data points by nonlinear regression (see Materials and Methods) were: 210 nM (SSAO), 1·3 mM (MAO-A) and 5·6 mM (MAO-B). In order to examine the variability in the sensitivity to procarbazine of tissue homogenates from individual animals, four separate homogenates were incubated with procarbazine (210 nM). SSAO activity was reduced to 54·6 $\pm$ 0·5% (n = 4) of the activity in control samples, preincubated with water. This very small variation was also seen in the results from pooled homogenates suggesting that little inter-individual variability exists in the sensitivity of rat brown fat SSAO to procarbazine. Reduc-

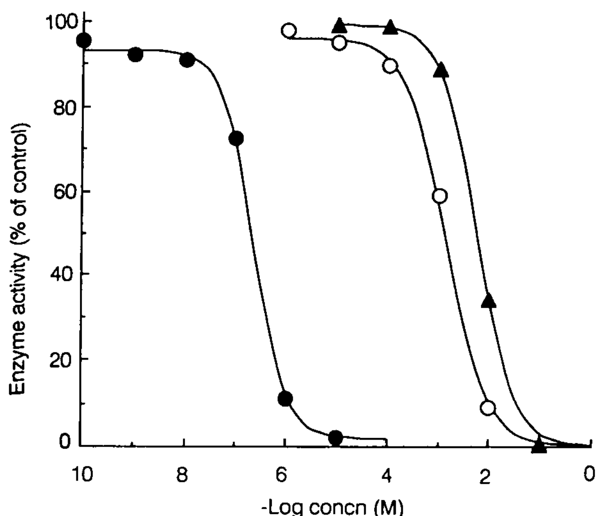

FIG. 1. Effect of procarbazine on brown adipose tissue SSAO (●), liver MAO-A (○) and MAO-B (▲) activities of the rat. Samples of homogenates were pre-incubated with procarbazine for 3 h at 37°C before addition of radiolabelled substrates. Deaminating activities are expressed as a percentage of control sample pre-incubated without inhibitor. Each point is the mean of 3 determinations, each in triplicate, on homogenates of pooled tissues.

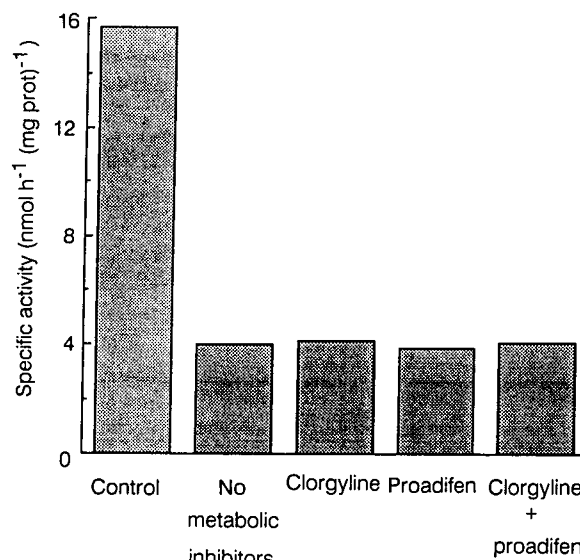

FIG. 2. Effects of clorgyline and proadifen on the inhibition of [^{14}C]benzylamine metabolism by procarbazine in rat brown adipose tissue homogenate. Following an initial pre-incubation with clorgyline, proadifen or a combination of both inhibitors, for 30 min at 37°C, samples of homogenates were pre-incubated further with procarbazine for 1 h at 37°C before addition of labelled substrate. Enzyme activities are shown together with values for controls and for samples pre-incubated with procarbazine only. Each value is the mean of 2 determinations, each in triplicate, on homogenates of pooled tissues.

tion of the pre-incubation time with procarbazine to 30 min resulted in a tenfold loss of potency against SSAO, with little change in the inhibition of MAO-A or -B (results not shown). In either case, a considerable degree of selectivity for SSAO over both forms of MAO is evident. Preliminary experiments had shown that no inhibition of SSAO occurred in samples which were not pre-incubated. This dependence of the degree of inhibition on time of pre-incubation suggested that conversion of procarbazine to an active species might be necessary before inhibition of SSAO could occur.

The role of metabolic enzymes in the formation of an active species

The primary metabolite of procarbazine circulating in the plasma of both rats and human subjects is azoprocarbazine (*N*-isopropyl-α-(2-methylazo)-*p*-toluamide) (Baggiolini et al 1969; see Tweedie et al 1987 for review) and the conversion of procarbazine to azoprocarbazine is brought about by MAO or cytochrome P450 (Coomes & Prough 1983). Brown fat is known to contain MAO (Barrand & Callingham 1982) and, although cytochrome P450 has not been detected (Sekhar et al 1990), some isozymes are present in the white fat (Drago et al 1982; Zyirek et al 1987) that contaminates the interscapular fat pad. Therefore, inhibitors of these enzymes were used to prevent the formation of azoprocarbazine and subsequent metabolites. Tissue homogenates were pre-incubated with clorgyline, proadifen or a mixture of both compounds, before pre-incubation with procarbazine. Fig. 2 shows that SSAO activity was reduced to 25% of the control value by 1 μM procarbazine. Inhibition of either, or both, MAO and cytochrome P450 had no effect on the degree of inhibition produced by procarbazine. Neither clorgyline nor proadifen had any effect on SSAO activity at the concentrations used.

These results did not rule out the possibility that another enzyme responsible for the conversion of procarbazine to an active species might be present. The rate of formation of an active inhibitor would be reduced if a more dilute homogenate containing less activating enzyme was used. To examine this possibility, homogenates of brown fat (1:40 and 1:200 w/v) were pretreated with clorgyline and proadifen before incubation with a submaximal concentration of procarbazine (2 μM) for 1 h. Remaining SSAO activity was determined and expressed as a percentage of activity in control samples pre-incubated without procarbazine. Mean values from 2 experiments assayed in triplicate were 5·1 and 6·0% for the 1:40 and 1:200 homogenates, respectively. The level of inhibition produced from 2 μM procarbazine in a 1:200 homogenate would be expected to approximate to that produced from 0·4 μM procarbazine in a 1:40 homogenate. However, no difference was seen between samples of either homogenate, suggesting that any activation of procarbazine is likely to be due to SSAO itself.

Effect of procarbazine on kinetic constants for [^{14}C]benzylamine deamination

Assays of [^{14}C]benzylamine metabolism (2–20 μM) were carried out in the presence of 1 μM procarbazine, both after pre-incubation for 90 min and without pre-incubation. A parallel experiment used water in place of inhibitor, with no pre-incubation. Fig. 3 shows a Hanes-Woolf plot of the results. The common intercept on the abscissa is indicative of a non-competitive interaction between procarbazine and SSAO. The inhibition obtained in homogenates without any pre-incubation can be attributed to interaction between procarbazine and the enzyme during the incubation with substrate for 10 min for the assay of enzyme activity.

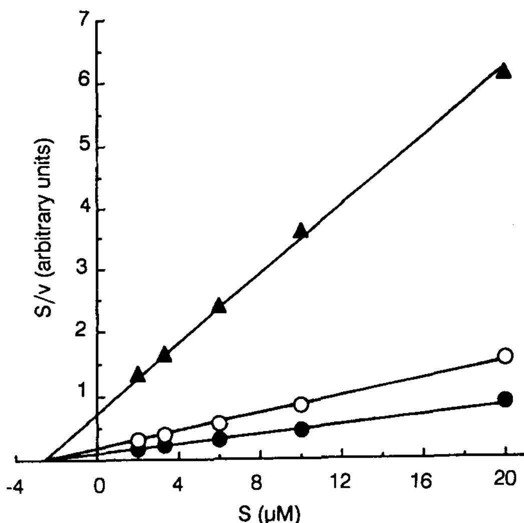

FIG. 3. Hanes-Woolf plot showing inhibition of [^{14}C]benzylamine metabolism by procarbazine in rat brown adipose tissue homogenates. Substrate concentration is represented as S and reaction velocity as v. Final procarbazine concentrations were 0 μM (●), 1 μM with no pre-incubation of samples before addition of substrate (○) and 1 μM with pre-incubation of samples for 90 min at 37°C before addition of substrate (▲). Each point is the mean of 2 determinations, each in triplicate, on homogenates of pooled tissues.

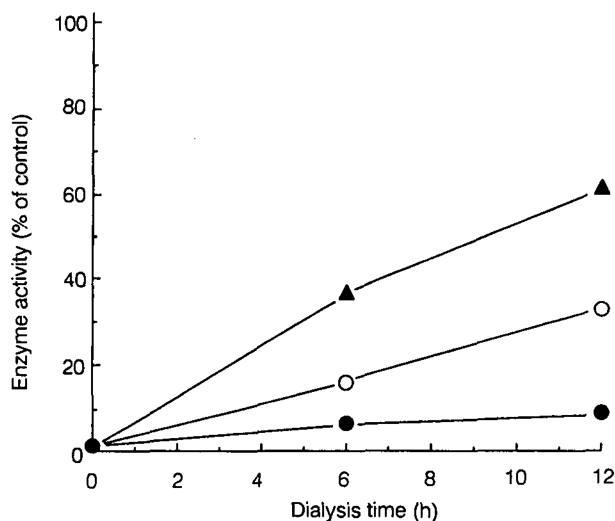

FIG. 4. Effect of temperature on the rate of recovery of [^{14}C]benzylamine-metabolizing activity in rat brown adipose tissue during dialysis, following addition of procarbazine. Samples of homogenates were pre-incubated with procarbazine for 2 h at 37°C and then dialysed at 4 (●), 25 (○) or 37°C (▲) before addition of substrate. Remaining enzyme activities are expressed as a percentage of those in control samples pre-incubated without inhibitor. Each point is the mean of 2 determinations, each in triplicate, on homogenates of pooled tissues.

Rates of reversal of inhibition at different dialysis temperatures

Dialysis experiments were performed to determine the characteristics of reversibility of inhibition. Fig. 4 shows the effects of dialysis against 1 L of 1 mM potassium phosphate buffer, pH 7·8 at 4, 25 or 37°C on enzyme activity after inhibition by 10 μM procarbazine. For all samples, a single buffer change was made after 3 h. Levels of inhibition in non-dialysed samples did not change over the 12 h dialysis period. Following dialysis for 24 h at 37°C, SSAO activity had returned to 77% of the control level (result not shown). These results indicate that, even at 37°C, inhibition of SSAO by procarbazine is only slowly reversible by dialysis.

Determination of the site of action of procarbazine by protecting the active site with excess substrate

In an attempt to determine whether or not inhibition of SSAO by procarbazine is due to an interaction at the active site of the enzyme, tissue homogenates were pre-incubated with procarbazine in the presence or absence of a high concentration of unlabelled benzylamine. After removal of benzylamine and unbound procarbazine, remaining enzyme activity was determined and the results expressed as percentages of the activities in control samples pre-incubated without procarbazine. Mean values from 2 experiments, assayed in triplicate, were 26·7% (procarbazine alone) and 82·1% (procarbazine in the presence of benzylamine). Control samples pre-incubated with benzylamine did not differ in activity from control samples pre-incubated with buffer (result not shown). This result suggests that benzylamine can decrease the binding of procarbazine to the enzyme during pre-incubation and that procarbazine may therefore interact with SSAO at the active site.

Discussion

In the present study, we have examined the effects of the benzylhydrazine derivative, procarbazine on rat amine oxidase activities in-vitro. This follows the observation by Lewinsohn et al (1978) that procarbazine showed greater potency against various human and rat SSAO activities than did a number of established SSAO inhibitors, with IC50 values lying in the high nanomolar range. Perhaps this was not surprising since hydrazine derivatives generally rank among the most potent of inhibitors of SSAO enzymes (see Lyles 1984 for review) and any search for a novel, selective SSAO inhibitor might begin within this extensive family of compounds. However, we chose to examine procarbazine more thoroughly because, although most hydrazine derivatives are too toxic for clinical use, procarbazine is employed in high doses, both in the treatment of Hodgkin's lymphoma (Bonadonna et al 1969) and of CNS tumours in children (van Eys et al 1985).

Following preliminary experiments which established that a pre-incubation period of between 2 and 3 h was necessary for procarbazine to reach full potency against SSAO, we have demonstrated that inhibition is dose-dependent and exhibits an IC50 against SSAO of rat brown fat of 210 nM. A comparison with IC50 values for inhibition of rat liver MAO activities shows a degree of selectivity for SSAO of some 4 orders of magnitude, particularly with respect to MAO-B. Should this also be the case in-vivo, then it may be possible to inhibit SSAO activity fully, without affecting MAO. Agents such as phenelzine have been shown to possess similar selectivity in-vitro; however, in in-vivo experiments, substantial inhibition of MAO occurs (Andree & Clarke 1982). Furthermore, it is possible that clinical doses of procarbazine, which are reported to inhibit MAO (De Vita et al 1965),

will also cause complete inhibition of SSAO enzymes. The possible consequences of such inhibition for the whole animal or patient remain unclear.

The observation that a decrease in potency against SSAO was seen when pre-incubation time was reduced suggests that conversion of procarbazine to an active metabolite takes place. No change in potency against MAO was seen. However, it is likely that inhibition of biogenic amine metabolism by MAO in-vivo occurs because procarbazine is itself a substrate for this enzyme (Coomes & Prough 1983). In these experiments therefore, the duration of pre-incubation would not be expected to alter the degree of inhibition unless substantial metabolism of procarbazine occurred during pre-incubation before addition of radiolabelled substrate. The product of procarbazine metabolism by MAO, and by cytochrome P450, is azoprocarbazine and the possibility that this compound might be the putative active species was investigated by employing the metabolic inhibitors, clorgyline (Johnston 1968) and proadifen (Cooper et al 1954). Concentrations of these inhibitors sufficient to abolish the activity of either group of enzymes had no effect on the potency of procarbazine against SSAO, suggesting that suicide (mechanism-based) inhibition of SSAO might be responsible. However, it was first necessary to rule out the involvement of some other converting enzyme.

Under normal circumstances, the degree of inhibition of an enzyme by an inhibitor is dependent upon inhibitor concentration but remains independent of the enzyme concentration. This is not the case when the inhibition of MAO-A in rat heart by clorgyline is considered, since the number of clorgyline molecules required to produce complete inhibition approximates to the number of active sites of the enzyme in the sample used (Fowler & Callingham 1979). Procarbazine does not fall into this category, since we have examined other compounds which display much greater potency against rat brown fat SSAO than does procarbazine (Holt et al 1992). Therefore, by diluting the homogenate containing the SSAO enzyme and consequently any converting enzyme by the same degree, the concentration of active metabolite should be reduced and this should be mirrored in the resultant degree of inhibition of SSAO. Experiments based on this principle were unable to show any effect due to dilution and it would appear, therefore, that procarbazine is a suicide inhibitor of SSAO in-vitro.

Kinetic experiments showed the interaction between SSAO and procarbazine to be non-competitive in nature. It is thought that hydrazine derivatives can interact with a carbonyl group on the cofactor, perhaps resulting in the formation of a diimide (Suva & Abeles 1978), and it is probable that procarbazine acts in a similar fashion. Our results suggest that in the first instance, affinity of procarbazine for the enzyme is low. However, an initial interaction with the cofactor could result in formation of an inhibitor-cofactor complex. Dialysis techniques, used to examine the nature of the binding involved, indicated that the adduct formed was not stable and therefore that tight binding, rather than a pure covalent interaction, probably occurred. Nevertheless, the resistance of this adduct to dialysis means that procarbazine can be considered irreversible for most in-vitro experiments. Potent, irreversible inhibitors of SSAO are already available, such as (E)-2-(3,4-dimethoxyphenyl)-3-fluoroallylamine (MDL 72145) (Lyles et al 1987) and hydralazine (Lyles 1984). However, the former also inhibits MAO-B (Zreika et al 1984) and the latter is relatively non-selective in its effects (Baker et al 1985). Procarbazine, unlike hydralazine, is also a relatively stable compound in aqueous solution and chemical degradation is sufficiently slow that solutions can be used up to a week after preparation, if stored at 4°C. It is possible, however, that hydracarbazine (6-hydrazino-3-pyridazine carboxamide), an antihypertensive agent closely related to procarbazine and hydralazine, could combine the desirable properties of both these compounds to become both stable and irreversible. Furthermore, the anticancer agent, dacarbazine (5-(3,3-dimethyl-1-triazeno) imidazole-4-carboxamide) may also behave in a similar manner.

It is likely that the cofactors of SSAO isolated from various sources are situated at the active sites of these enzymes (Suva & Abeles 1978; Hartmann & Klinman 1990, 1991; Janes et al 1990; Janes & Klinman 1991). Thus the ability of benzylamine to afford substantial protection against inhibition by procarbazine suggests that, in rat brown fat, the cofactor is at the active site. It would seem possible that this protection is the result of Schiff's base formation between procarbazine and benzylamine. However, not all the protection may be due to this interaction since the rate of binding of [^{3}H]hydralazine, which does not contain a carbonyl group, to partially purified rat brown fat SSAO is reduced when incubation is performed in the presence of benzylamine (Barrand & Callingham 1985). Until recently, much uncertainty surrounded the identity of the cofactor in SSAO enzymes. However, recent evidence points towards 6-hydroxydopa as the cofactor in SSAO from bovine plasma (Janes et al 1990). The inhibition of this enzyme by procarbazine has not yet been examined. However, the similarities in IC50 values obtained for procarbazine against rat brown fat SSAO in our laboratory and against SSAO enzymes from various human tissues and rat lung by other workers (Lewinsohn et al 1978), provide tentative evidence that conservation of the cofactor may occur between these enzymes.

In patients receiving procarbazine, many toxic side effects have been observed, including nausea, vomiting and immunosuppression (see Tweedie et al 1987). An ability to cross the blood-brain barrier (Oliverio et al 1964) means that central neurotoxicity is not uncommon and this has been attributed, at least in part, to the competitive inhibition of MAO (Andree & Clarke 1982; Pfefferbaum et al 1989). Since the drug is used widely in children, it is particularly important to prevent such unpleasant psychotic disturbances and to this end, a low tyramine diet is often given for the duration of the treatment (Pfefferbaum et al 1989). Recently, Elliott et al (1989), using the isolated perfused mesenteric arterial bed of the rat, have shown that inhibition of either MAO-A or SSAO alone had no effect on the pressor response to tyramine. However, when both enzymes were inhibited together, significant increases were seen both in the maximum pressure attained and in the area under the curve of the response to low and high doses of the amine. Similar results have also been obtained when tryptamine was applied to rat aorta preparations in-vitro (Taneja & Lyles 1988). Such a situation might arise after administration of procar-

bazine in clinical doses. While this is not necessarily the case in-vivo, it is important to remember that other enzymes besides MAO might contribute to these side effects. For instance, hydralazine is known to affect several enzymes involved with the turnover of biogenic amines, such as dopamine-β-hydroxylase (Liu et al 1974) and it would be surprising if procarbazine did not have some effect on other such enzymes.

Any attempt to explain the neurotoxic disturbances in patients receiving procarbazine is complicated further by the possible involvement of methylamine. This endogenous amine is known to be a substrate for SSAO, both in-vitro (Precious et al 1988; Lyles et al 1990) and perhaps in-vivo (Lyles & McDougall 1989). Cytotoxic actions of methylamine have been demonstrated on cultured neurones and fibroblasts (Gilad & Gilad 1986). In uraemic patients, raised plasma methylamine levels may contribute to neurological disorders seen in these subjects (Simenhoff 1975; Baba et al 1984). The administration of semicarbazide or hydralazine to rats enhances the daily urinary excretion of methylamine (Lyles & McDougall 1989) and [^{14}C]methylamine has been recovered from the urine of rats treated with [^{14}C]procarbazine (Schwartz 1966). These results suggest that, in rats at least, blood levels of this neurotoxic amine might rise after procarbazine administration, both because metabolism of endogenous methylamine is prevented and because methylamine is itself a metabolite of the drug.

While no natural substrate and consequently no physiological role has been identified for most SSAO enzymes, some evidence exists for an involvement in tissue growth and renewal (Pierce et al 1990). Human plasma SSAO activity is known to be reduced in conditions of rapid tissue growth such as in patients with severe burns or with solid tumours (Lewinsohn 1984). This might be due to many factors but, in sheep, plasma SSAO activity is known to be susceptible to hormonal regulation (Elliott et al 1991). Thus the possibility exists that hormones released from certain tumours could influence the activity of SSAO enzymes. The use of an anticancer agent which is also a powerful inhibitor of SSAO enzymes might therefore have unforeseen consequences. The present findings suggest that procarbazine, through its relatively selective inhibition of SSAO activities, may well prove useful in the search for the physiological role played by these enzymes.

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