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SHORT COMMUNICATIONS

Potent inhibition of diamine oxidase with the hydroxybenzyloxamines NSD-1039, NSD-1531 and NSD-1024

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In cells, the precise role of DAO* (EC 1.4.3.6) is unknown, but its activity is implicated in the regulation of cell proliferation [1] and tissue growth [2]. Putrescine, a diamine and therefore DAO substrate, is the precursor for polyamine biosynthesis in mammalian cells. Polyamines are able to influence the biosynthesis of proteins, RNA and DNA, and are a prerequisite for cell proliferation mechanisms [3].

Putrescine is secreted by cells. Extracellular enzymic oxidation of diamines results in cytotoxicity [4] at least *in vitro* and so could be involved in unresolved pathological processes, especially cystic fibrosis [5] and damage to those tissues rich in DAO such as the liver, placenta, kidney, platelets [6] and, curiously, tumours [7].

DAO has pyridoxal-phosphate as its co-factor. Since the hydroxybenzyloxyamines NSD-1055 and NSD-1024 inactivated pyridoxal-P on histidine decarboxylase [8], NSD-1024 and two related compounds NSD-1039 and NSD-1531 (structures in Fig. 1) were examined for DAO inhibitory activity.

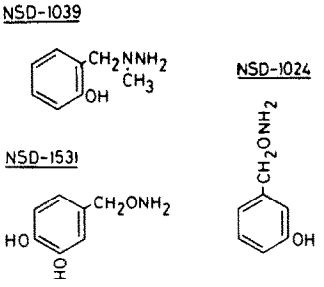

Fig. 1. Structure of NSD-series drugs.

Methods. The DAO radiochemical assay used has been described elsewhere [4]. Briefly, in a reaction-mixture the [^{3}H]+putrescine^{2+} is converted by DAO to radiolabelled 4-aminobutyraldehyde^{1+} (Fig. 2), both unreacted substrate and labile primary product (half-life 2.0 hr) are separated by ion-exchange chromatography and measured. The ability of NSD-series drugs to inhibit such conversion was assessed.

Results. All three NSD drugs tested were potent inhibitors of DAO activity with a relative efficacy NSD-1039 > NSD-1531 > NSD-1024 (data in Fig. 3). Further investigation showed that the drug inhibition was irreversible.

The radiochemical assay used supersedes that previously employed for routine assay of DAO activity, namely the measurement of $^1\Delta$-pyrroline [9].

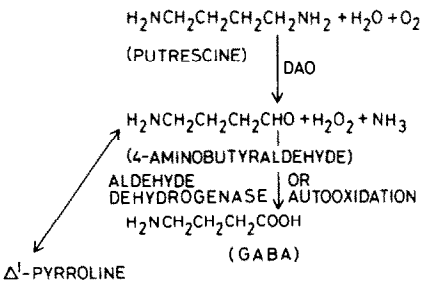

Fig. 2. Catabolism of putrescine [20] producing cytotoxic 4-aminobutyraldehyde and by-products [4].

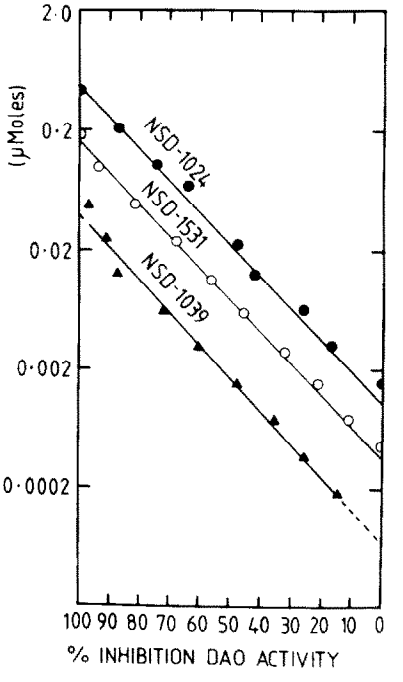

Fig. 3. Inhibition of DAO activity by NSD-series drugs as assessed by radiochemical assay. Reaction-mixture consisted of 0.033 U of hog kidney DAO and 0.01 μmoles [^{3}H]putrescine in 2.0 cm^3 P-buffered saline at pH 7.2 for 2 hr at 37°. Results shown were obtained by the measurement of [^{3}H]putrescine depletion; similar results (not shown) were obtained by measurement of [^{3}H]-4-aminobutyraldehyde formation. Hog kidney DAO (Sigma Ltd,) where 1.0 U oxidized 1.0 μmole of putrescine hr^{-1} at pH 7.2 at 37° by $^1\Delta$-pyrroline measurement.

* Abbreviations used: DAO, diamine oxidase; PAO, polyamine oxidase; P, phosphate; GABA, γ-aminobutyric acid.

Aminoguanidine, sodium semicarbazide and a few other less effective agents have long been recognized as DAO inhibitors [10], and recently 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, metal ions [11] and 1,1′-tetramethylene diguanidine [12] have been reported as inhibitory. The anticancer drug methyl-glyoxal *bis*(guanylhydrazone) also inhibits DAO [13] and has a multiplicity of pharmacological actions which culminate in cytostasis [14]. It formed a precipitate together in solution with phosphate salts but not with pyridoxal-P, so was unlikely to inhibit enzyme activity by enzymic pyridoxal-P inactivation. Nonetheless, evidence presented to date is not sufficient to establish whether methyl-glyoxal *bis*(guanylhydrazone) inhibits by reacting with pyridoxal-P.

Incidentally, assay of ruminant serum PAO which is frequently reported as containing a flavin co-factor [15] when, in fact, it is undoubtedly pyridoxal-P [16], was also potently inhibited by the NSD-series drugs [17]. Mammalian liver PAO has flavin as its co-factor [18] so should not be susceptible to inhibition by the NSD-series drugs. The co-factor for human serum PAO found during pregnancy [19] has not been ascertained.

In summary, NSD-1039, NSD-1051 and NSD-1024 were potent *in vitro* inhibitors of DAO, as well as certain PAO's. If products of diamine enzymic oxidation prove to be either causative or symtomatic of any tissue disease of clinical importance, then there would be a need for administration of suitable DAO inhibitor drugs. Additionally, such drugs including perhaps those of the NSD-series could be invaluable tools in the currently rapidly expanding field of polyamine research in cell biology and pharmacology.

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