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THE INHIBITION OF AMINE OXIDASE AND SPERMINE OXIDASE BY AMIDINES

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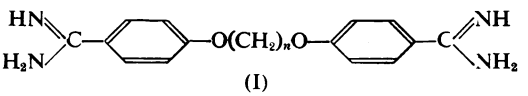
It has been known for some time that the homologous series (I)



(I)

includes substances which are strong inhibitors of the amine oxidase of rabbit liver (Blaschko and Duthie, 1945). The two most active members of the series are propamidine ($n=3$) and pentamidine ($n=5$).

While experimenting with a preparation of the enzyme from guinea-pig liver, it was recently noted that this enzyme was much less strongly inhibited by pentamidine. We have therefore examined all the available members of the homologous series with preparations of amine oxidase from rabbit, guinea-pig, dog, cat, sheep, pig, ox, squid and sea-urchin. The two invertebrates were chosen as representatives of phyla in which amine oxidase is present. The inhibitory action of the amidines upon the spermine oxidase of sheep and ox serum was also studied.

METHODS

Most of the amidines tested were the original samples obtained in 1945 from Dr. A. J. Ewins, F.R.S.; in addition, the octamethylene derivative as the diisethionate, was made available through the kindness of Dr. R. Wien, of Messrs. May and Baker.

The source of mammalian amine oxidase was liver; we also used pig kidney in order to compare enzyme from two different tissues in the same species.

The amine oxidase preparations were fresh extracts, dialysed against tap water, with sodium phosphate buffer of *p*H 7.4 added in the manometer flask. The livers of the squid (*Loligo forbesii*) and the gonads of the sea-urchin (*Echinus esculentus*) were from animals dissected in Plymouth six months earlier and kept frozen at $-10°$. All the extracts were made up before dialysis, so that 1 g. of wet tissue was present in 2 ml. In order to obtain suitable manometer readings, different volumes of the tissue preparations were used in different experiments: they varied from 0.15 ml. in one of the experiments with ox liver to 1.4 ml. with sea-urchin gonads. Oxygen uptakes in the first 15 min. differed from 20.5 μl. in one experiment with squid liver to 135 μl. in one experiment with pig kidney.

The spermine oxidase preparations were ox or sheep serum, dialysed against 0.067M-sodium phosphate buffer of *p*H 7.4.

In the manometric experiments, the main compartment of the manometer flasks contained the enzyme preparation, brought to a total volume of 1.6 ml. by adding sodium phosphate buffer; the side arm contained 0.2 ml. of substrate (0.1M-tyramine hydrochloride or 0.05M-spermine tetrahydrochloride) plus 0.2 ml. of either water or inhibitor. An "enzyme blank" was also included. The gas phase was oxygen and the temperature was 37.5°.

For the calculation of the percentage inhibitions, the 0 to 15 min. readings were used when tyramine was the substrate; in the experiments with spermine the calculation was based on the 5 to 25 min. readings. During these periods the reaction rates remained approximately linear.

RESULTS

The results are shown graphically in the twelve diagrams of Fig. 1. Each of the diagrams shows the source of enzyme, the molar concentrations of the inhibitors used, and percentage inhibitions obtained. Each point represents one result.

The results, as shown in the diagrams, may be summarized as follows:

(*a*) The inhibition of amine oxidase by the amidines tested differs from species to species. Thus, for example, the amine oxidase of the rabbit

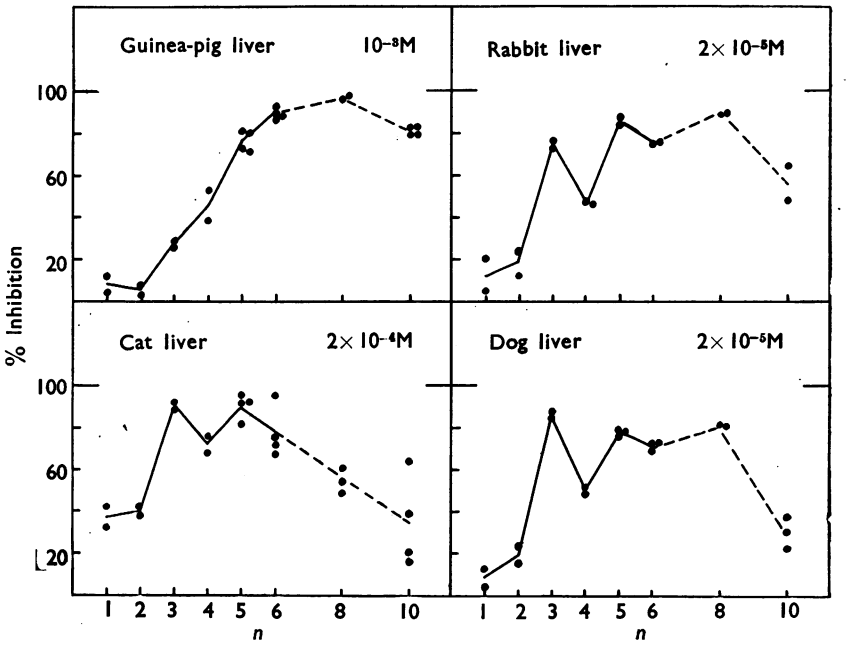

Guinea-pig liver
10⁻⁸M
Rabbit liver
2× 10⁻⁵M
Cat liver
2× 10⁻⁴M
Dog liver
2× 10⁻⁵M
% Inhibition
100
60
20
1 2 3 4 5 6 8 10
n

FIG. 1a

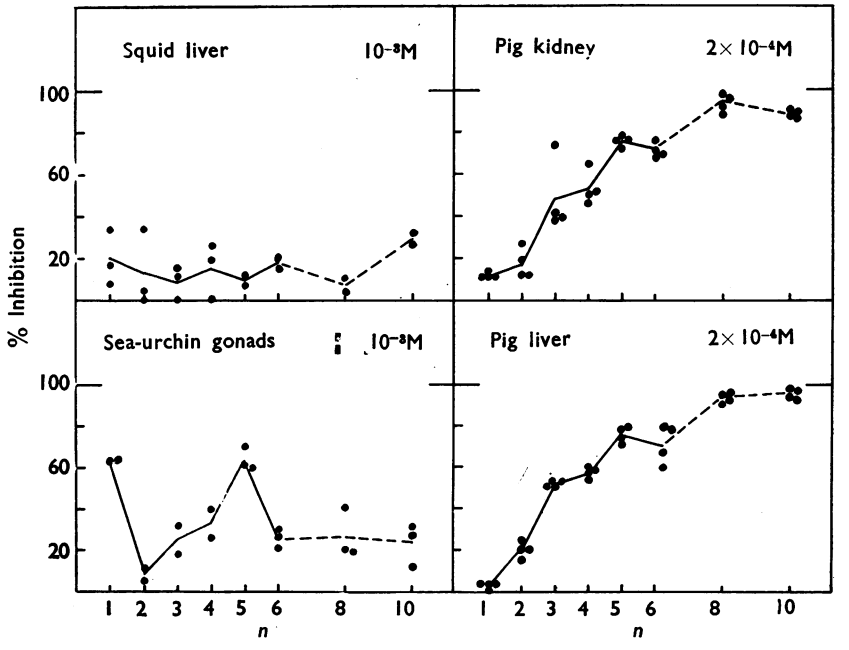

Squid liver
10⁻⁸M
Pig kidney
2× 10⁻⁴M
Sea-urchin gonads
10⁻⁸M
Pig liver
2× 10⁻⁴M
% Inhibition
100
60
20
1 2 3 4 5 6 8 10
n

FIG. 1b

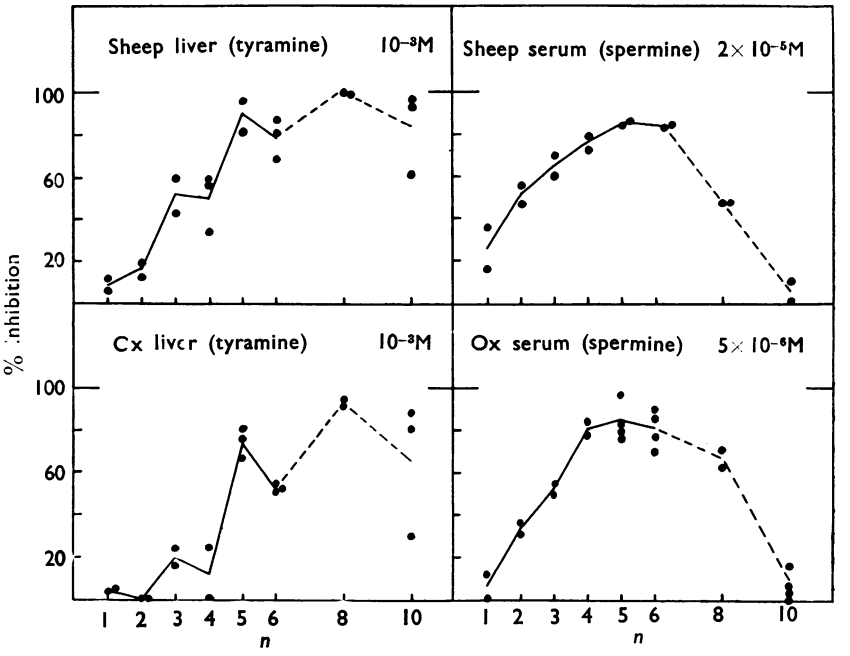


FIG. 1c

FIG. 1.—Inhibition of amine oxidase and of spermine oxidase by diamidines of the homologous series (I). Ordinates, percentage inhibition; abscissae, number (*n*) of carbon atoms in the polymethylene chain. Each diagram gives the source of enzyme and the molar concentration of inhibitor used. In all diagrams in Fig. 1a and 1b the substrate was tyramine; in those of Fig. 1c the substrate used is indicated in parentheses.

and of the dog (Fig. 1a) is very sensitive, whereas that of the guinea-pig (Fig. 1a) and of the squid (Fig. 1b) is rather insensitive.

(*b*) The relation between chain length and inhibitory activity also differs from species to species. The experiments with rabbit liver are in good agreement with the findings of Blaschko and Duthie (1945); the optima for $n=3$ and $n=5$ are again evident, and there appears to be a third optimum in the neighbourhood of $n=8$. The curve for dog liver is rather similar to that for rabbit liver (Fig. 1a). With pig (Fig. 1b) and guinea-pig (Fig. 1a) liver the long-chain members are more active than those with short chains. Enzyme from sea-urchin gonads has one optimum for $n=1$ and one for $n=5$ (Fig. 1b).

(*c*) Little difference was noted between the inhibitions of pig liver enzyme and pig kidney enzyme. Not only was there no significant difference between the percentage inhibitions but also the curves relating inhibition to chain length were very similar (Fig. 1b).

(*d*) Spermine oxidase, from ox and from sheep serum, was strongly inhibited by amidines (Fig. 1c); the optimum chain length with both preparations was in the region of $n=4$ to $n=6$, in marked contrast to the results with amine oxidase from these two species (Fig. 1c).

DISCUSSION

These results show that the amine oxidases from different species are different entities with differing inhibitor specificities. The results with pig tissues suggest that, at least in this species, enzyme from two different organs is not distinct. On the other hand, the two different enzymes from ruminants—amine oxidase and spermine oxidase—display entirely different inhibitor specificities within the same species.

SUMMARY

1. The inhibition of amine oxidase and of spermine oxidase from different species by members of a homologous series of diamidines has been studied.

2. Enzyme preparations from different species display marked differences in their sensitivity to amidines; moreover, the relation between the length of the polymethylene chain and inhibitory activity differs from species to species.

3. Spermine oxidase is strongly inhibited by some of the diamidines studied.

REFERENCE

Blaschko, H., and Duthie, R. (1945). *Biochem. J.*, **39**, 347.