THE PRESENCE OF ANTIBODIES TO HUMAN DOPAMINE-β-HYDROXYLASE IN COMMERCIALLY AVAILABLE ANTISERA

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Received September 27,1974

SUMMARY

Three criteria have been used to demonstrate the presence of antibodies to human dopamine-β-hydroxylase in commercially available antisera directed against various human serum fractions. These criteria are the inhibition of enzyme activity, complement fixation and binding of ^{125}I-labelled dopamine-β-hydroxylase to the immobilized antisera. The level of antibody present in some of these antisera was sufficient to allow their use in the radioimmunoassay of the enzyme. The possibility of other useful antibodies occurring in these and similar antisera is suggested.

The development of techniques such as radioimmunoassay and affinity chromatography has led to the preparation of antibodies against a wide variety of antigens. Such studies generally use lengthy procedures for purification of the antigen and involve a considerable wait for the antibody to be produced. It should be noted that many immunological procedures do not require a monospecific antiserum and in some laboratories multiple immunizations have been used routinely (1,2).

Dopamine-β-hydroxylase is normally present in human serum. It therefore follows that it will be present in one or more of the fractions obtained when serum proteins are crudely fractionated into various classes. Since dopamine-β-hydroxylase is highly antigenic (3) it was conceivable that injection into goats of serum fractions containing this enzyme even as a minor contaminant, would result in the production of antibodies against both this enzyme as well

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as against the major proteins in the fraction. We wish to report the detection of human dopamine-β-hydroxylase antibodies in commercially available goat antisera prepared against human serum fractions.

MATERIALS AND METHODS

Goat antisera directed against specific human serum fractions were obtained from Miles (U.S.A.). Crude dopamine-β-hydroxylase was prepared from human adrenal glands obtained at autopsy. The glands were cut into small sections and placed in 5 volumes of ice-cold 0.02 M phosphate buffer, pH 7.4. The sections were homogenized in a Waring Blender for 30 sec and then centrifuged at 30,000 x g for 1 hr. The supernatant was fractionated with ammonium sulfate and the fraction precipitating between 25% and 40% saturation was reconstituted with 0.02 M phosphate buffer, pH 7.4, and dialyzed overnight against the same buffer. After dialysis the preparation was again centrifuged at 30,000 x g for 1 hr. to remove a small amount of insoluble material. The supernatant was frozen and stored in aliquots at -20°. This fraction had a specific activity of 4 mμmol of octopamine formed per min per mg protein and was used as the source of human enzyme in the inhibition studies. Inhibition of the dopamine-β-hydroxylase activity by each antiserum was determined after incubation of the enzyme with the corresponding antisera for 5 minutes at 37°.

A more purified form of the enzyme was prepared for use in the complement fixation experiments. A single human adrenal gland, obtained at autopsy, was homogenized in 0.3 M sucrose and the chromaffin vesicles were isolated by differential and density gradient centrifugation (4). These vesicles were lysed in 0.02 M sodium acetate buffer, pH 6.5, recentrifuged to remove insoluble material and dialyzed overnight against the same buffer. The specific activity of this preparation was 280 mμmol of octopamine formed per min per mg protein. This lysate was stored until required at 2° in the presence of 0.1% sodium azide.

Dopamine-β-hydroxylase was assayed by the photometric assay of Nagatsu and Udenfriend using tyramine as substrate (5). Microcomplement fixation was performed by the method of Schneider et al. (6). The ability of the antisera to bind ^{125}I-labelled dopamine-β-hydroxylase was determined by first binding each antiserum to 0.5 micron polystyrene beads by the procedure described previously (7), followed by incubation of these coated beads with the ^{125}I-labelled enzyme. ^{125}I-labelled human dopamine-β-hydroxylase was prepared as described elsewhere (8).

RESULTS

Inhibition of dopamine-β-hydroxylase activity

One criterion for the presence of antibody to dopamine-β-hydroxylase is the inhibition of enzyme activity. The inhibition obtained with 10 μl of commercial antisera directed against a number of serum fractions is shown in Table I. Inhibition of enzyme activity occurred with antisera against all the fractions except for the one directed against albumin. The commercial antisera against serum glycoprotein and haptoglobin fractions were the most inhibitory. No

TABLE I

INHIBITION OF HUMAN DOPAMINE-β-HYDROXYLASE ACTIVITY BY VARIOUS GOAT ANTISERA DIRECTED AGAINST HUMAN SERUM FRACTIONS

Antiserum Directed Against	Reported Titer*	Percent Inhibition**
Haptoglobin	3.7	51
α_1-Glycoprotein	0.85	42
α_2-Macroglobulin	2.1	27
Whole human serum	-	21
Pre-albumin	1.0	17
α_1-Trypsin inhibitor	1.7	15
Albumin	6.1	0
Normal goat serum	-	0
Bovine dopamine-β-hydroxylase	-	31
Human dopamine-β-hydroxylase	-	86

Crude human adrenal dopamine-β-hydroxylase was preincubated at 37° for 5 min. with 10 µl of the appropriate antiserum before assay. The dopamine-β-hydroxylase present in each tube was able to form 76 mµmoles of octopamine in 20 min.

*Titer is expressed as mg of precipitable antibody per ml.

**These values cannot be directly compared since at 10 µl the response is not linear (see Fig. 1).

significant inhibition of enzymatic activity was observed when normal goat serum was used. Rabbit antiserum produced in this laboratory against pure dopamine-β-hydroxylase obtained from human adrenal glands showed the greatest inhibition of all the antisera tested.

Immunotitrations, using enzyme inhibition as an index, are shown in Fig. 1 for a commercial antiserum against human serum glycoproteins and for rabbit antiserum directed against a purified preparation of human adrenal dopamine-β-hydroxylase. A titration using normal goat serum is shown for comparison.

glycol TMS derivative emerged from the GC column (15 min). Relative abundances of ions of *m/e* 289, 291, and 293 were determined by calculating mean values obtained from ten successive mass spectral scans taken at the summit of the GC peak.

RESULTS AND DISCUSSION

The yield of the formed (20*R*,22*R*)-20,22-dihydroxycholesterol as determined from its ^{14}C content by liquid scintillation counting was approximately 600 ng in both experiments A and B. No significant radioactivity was found in the blank experiment. These results agreed with the intensities of the ions observed by mass spectrometry of the TMS derivatives; only background ions were seen in the blank experiment. That the isolated glycol arose almost exclusively from cholesterol and did not contain significant preformed material was also established in another experiment with the same adrenal enzyme preparation and [4-^{14}C]cholesterol except that only $^{16}O_2$ was used. In this experiment (to be described elsewhere) it was shown by mass spectrometry that the ^{12}C to ^{14}C ratio of the glycol and that of the added or reisolated cholesterol from the incubation were not significantly different.

The side-chain C_8 ion intensity distribution giving the abundance of the glycol TMS species containing $^{16}O_2$ (*m/e* 289), $^{16}O^{18}O$ (*m/e* 291), and $^{18}O_2$ (*m/e* 293) is given in Table I. Included in this Table is the oxygen composition of the atmosphere during the incubation as well as the calculated ion abundances for the two situations in which the two oxygen atoms of the glycol are derived either from two separate molecules or from the same molecule of oxygen. The ^{18}O content of the side-chain of the formed (20*R*,22*R*)-20,22-dihydroxycholesterol in experiments A and B was 90.5 and 68.0%, respectively, of that observed in the atmosphere during the incubation.

It is apparent from Table I that, whereas the oxygen atmosphere during the incubation had no significant $^{16}O^{18}O$ content, the recovered glycol con-

TABLE 2

COMPLEMENT FIXATION BY VARIOUS ANTISERA IN THE PRESENCE OF HUMAN DOPAMINE-β-HYDROXYLASE

Sample	Complement Fixed (Arbitrary Units)
Haptoglobin antiserum	10
α_2-Macroglobulin antiserum	20
Albumin antiserum	0
Dopamine-β-hydroxylase antiserum	640
Concanavalin A	0

Each analysis was performed in duplicate.

tested. However, the commercial antisera that showed the greatest ability to bind the labelled enzyme were those directed against the haptoglobin fraction and whole serum. It was further shown that the binding was inhibited by the purified human enzyme. Titration curves obtained with increasing amounts of purified enzyme were essentially the same for the commercial antisera directed against haptoglobin and whole serum as for the specific antiserum to human dopamine-β-hydroxylase.

DISCUSSION

In view of the recent findings that dopamine-β-hydroxylase is a glycoprotein (10) we suspected that we might detect antibodies to dopamine-β-hydroxylase in antisera directed against serum glycoproteins. The presence of antibodies to doapmine-β-hydroxylase in other antisera was surprising but may possibly be accounted for by the large size of the molecule and therefore its presumptive co-fractionation with the α_2-macroglobulin and haptoglobin fractions. Of primary significance is the fact that the titer of antibody present in a number

TABLE 3

BINDING OF ^{125}I-LABELLED HUMAN DOPAMINE-β-HYDROXYLASE

TO IMMOBILIZED ANTISERA

Antiserum to	Relative Binding Capacity
Dopamine-β-hydroxylase	100
Haptoglobin	44.5
Whole serum	29.8
α_1-Glycoprotein	19.2
α_1-Trypsin inhibitor	17.2
Pre-albumin	16.5
α_2-Macroglobulin	16.4
Albumin	10.1*
Bovine dopamine-β-hydroxylase	64.6

All antisera, unless otherwise indicated are to human proteins. Each analysis was performed in duplicate using 100 µl of the coated beads and 10,000 cpm of ^{125}I-dopamine-β-hydroxylase in a total volume of 1.2 ml. Incubation was at room temperature for 16 hours. The amount of binding that occurred with each antiserum is shown as a percentage of the amount bound by the specific antiserum to human dopamine-β-hydroxylase.

*This value represents the blank obtained in the radioimmunoassay.

of these antisera is sufficient to allow their use in the radioimmunoassay of the enzyme and possibly also in conjunction with more classical procedures, for the purification of dopamine-β-hydroxylase by affinity chromatography.

Three criteria were employed to establish the presence of antibody to dopamine-β-hydroxylase. Although the enzyme inhibition studies alone are insufficient to establish the presence of antibody, when combined with the results obtained from the complement fixation and antigen binding studies, the findings

clearly show that antibody to dopamine-β-hydroxylase is present in the commercial antisera. The fact that there is no quantitative correlation among the three parameters is not too surprising since antibodies have many binding sites which can vary independently from species to species and with the time and route of innoculation etc. (11). The titers of the commercial antisera were lowest measured by complement fixation. However, microliter quantities of the antisera were sufficient for any type of immunoassay for dopamine-β-hydroxylase.

Obviously to produce antibody to dopamine-β-hydroxylase, the serum fractions which were used to prepare the commercial antisera must have contained the enzyme protein. Only a glycoprotein fraction was available to us, obviously not the one used for the innoculation. The fraction did not exhibit detectable enzyme activity, which is not surprising since the commercial procedure for purification and storage may very well have destroyed the activity. However, enzyme protein was detected by immunoassay.

Dopamine-β-hydroxylase is only one of many enzymes which are normally found in human serum. The presence of antibodies to dopamine-β-hydroxylase in commercial antisera suggests that such antisera might serve as an excellent and readily available source for antibodies to other human enzymes as well.

We wish to thank Dr. Burness and Mr. Thomas Orr, N. J. College of Medicine, for their assistance with the complement fixation studies, and also Dr. Paul Thomas for his assistance in the production of antisera to the human and bovine dopamine-β-hydroxylases.

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