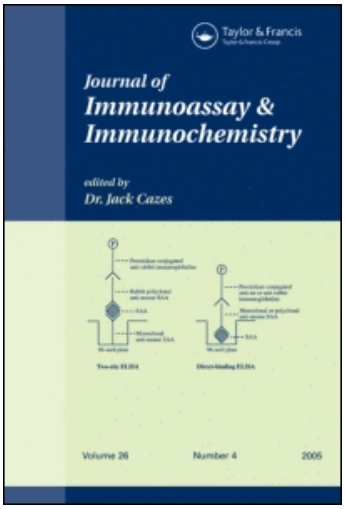

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Technical Note: Is there a Season for Iodinating Relaxin?

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Technical Note: Is there a Season for Iodinating Relaxin?

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ABSTRACT

We report a loss of antibody binding in our porcine relaxin radioimmunoassay that has occurred during the coldest periods of the past three winters. We do not know the reason for this seasonal variation but we have eliminated cold deterioration of the iodide used for radioiodination and equipment failures as explanations.

INTRODUCTION

Over ten years ago we developed a radio-immunoassay (RIA) for porcine relaxin (pRXN) at the University of Bristol in Britain (1) based upon the method of Sherwood et al.,(2). We have conducted analyses regularly with this RIA both at the University of Bristol, U.K. (~6 years) and more recently at the University of Guelph (~5 years) in Ontario, Canada. The assay is based upon antisera to purified pRXN raised in rabbits. During its operation at Bristol we experienced periods of difficulty with the assay. In particular there were times the binding of the iodinated pRXN to the antibody was poor. We did not keep systematic records of the binding at that

time, and we were resigned to the likelihood that the problems were intrinsic to the assay. It is known (3) that the relaxin molecule is sensitive to iodination which tends to interfere with its ability to bind to receptors. Thus, it seemed plausible that the apparent capriciousness of its binding to antibody in our RIA might be explained in the same way.

We encountered similar problems with binding when we set up the assay in Canada where it was performed by different technical staff using different equipment, thereby eliminating individual bias and equipment-related explanations. Over a four-year period however, we have noted that our problems recurred, but exclusively in the coldest months (December - March), whereas the assay worked satisfactorily during the rest of the year.

METHODS

We carried out iodinations according to the method of Bolton and Hunter (4), as modified by Kwok, McMurtry and Bryant (5) but with slight variations. A solution of 18.75μg purified porcine relaxin in 0.1M borate buffer at pH 8.5, was allowed to react on ice with 0.75μg 3-(p-hydroxyphenyl) propionic acid N-hydroxy-succinimide ester (Sigma Chemical Co., St.Louis, Mo., U.S.A.) for 40-45 minutes. The mixture was iodinated, using the chloramine-T method, with 37MBq iodine^{125} obtained from Amersham International, Amersham, Bucks.,U.K.). The radioactive iodine was shipped as sodium iodide in a dilute solution of sodium hydroxide. It had a specific activity of about 15.0 nCi (558.7.9 GBq per μg iodine), and the manufacturers estimate purity at 98.1%. The reaction mixture was diluted with

potassium iodide solution and transferred to a Sephadex G25 column (18X1 cm) equilibrated with 0.05M phosphate buffer at pH 7.5, containing 1% bovine serum albumin (BSA) (Sigma Chemical Co.,St.Louis, Mo.,U.S.A.). Two peaks of radioactivity were obtained and the most active tubes (3-4) from the first (protein) peak were transferred to eppendorf tubes in 100μl aliquots and stored frozen. On the day of use, the labelled iodine solution was purified further on a Sephadex G50 superfine column (18X1 cm) equilibrated with 0.05M barbitone buffer, pH 8.5, containing 10mg BSA/ml, from which it was eluted with more of the same buffer. Three peaks were obtained of which the middle one was of highly purified ^{125}I-labelled relaxin. This material, suitably diluted, was used in the RIA.

RESULTS

The data on antibody binding from our records of radioiodination are shown in Table 1.

It can be seen that whereas the specific activity of the radioiodinated pRXN remained relatively stable through successive iodinations over the period 1987-1991, the antibody binding fell below 15% only during the periods between November and March. Indeed, it appears that with the exception of 11/17/88 where the weekly maximum was +4°C, this only occurred when the external maximum temperature was 0°C or below. Since the temperature data were weekly maxima it is possible that on average the ambient temperatures were lower than those cited.

TABLE 1

Dates, peak counts(10^6 cpm/μl) of pRXN fraction, specific activities (μci/μg pRXN) and binding characteristics of Iodinated Porcine Relaxin (1987-1991) together with the weekly maximum and minimum environmental temperatures in degrees centigrade.

Date	Peak 10^6 cpm/10ul	uci/ug pRXN	Dilution	Antibody % binding	Weekly temp °F max	min
11/17/87	1.9	29.6	1:5000	22.7	+6	-6
04/08/88	3.4	38.4	1:6000	30.0	+6	-3
06/28/88	3.2	28.4	1:6500	27.7	+19	+8
11/17/88	1.2	32.6	1:6500	9.2	+4	-3
01/10/89	3.7	28.5	1:6500	10.1	-3	10
03/28/89	3.2	23.9	1:6400	8.4	-7	23
04/14/89	1.5	27.7	1:6000	26.4	+3	-7
06/06/89	4.6	39.8	1:5500	20.6	+19	+6
10/26/89	6.0	42.1	1:10000	21.2	+6	+1
01/11/90	6.0	27.2	1:6000	4.9	-2	-9
03/13/90	2.4	293.7*	1:6500	6.8)	-3	-14
03/14/90	1.6	25.7	1:6500	14.2)		
04/11/90	5.6	30.0	1:6000	32.0	+2	-5
04/11/90	1.7	31.8	1:6000	42.5	+2	-5
07/20/90	0.9	35.3	1:6500	28.2	+19	+9
11/01/90	0.9	33.3	1:6000	19.8	+9	-3
12/12/90	1.0	18.3	1:6000	14.5	0	-7
12/12/90	1.2	24.6	1:6000	14.5	0	-7
05/01/91	1.0	22.2	1:6000	36.6	+11	+2
09/24/91	1.1	29.1	1:5500	31.6	+16	0

Iodinations yielding antibody binding of less than 15% are shown in bold.

The weekly maximum and minimum temperatures were obtained from "Climatic Perspectives", a weekly review of the Canadian Climate published by Environment Canada; Volumes 9,10,11,12,13, (1987-1991).

*The specific activity of relaxin iodinated on 13 March 1990 is much higher than at other times because we departed from our usual protocol and iodinated 1.00μg relaxin instead of the usual 18.75μg in order to increase specific activity for a particular experiment.

DISCUSSION

The explanation of this seasonal variation in the effectiveness of antibody binding is obscure. With one exception (11/17/88) where the weekly maximum was +4°C, all the periods of poor binding occurred when the weekly maximum temperature was 0°C or below. The iodide is supplied with a warning by the manufacturers that it should be "stored at ambient temperature (15-25° C)" because storage near or below 0° C can result in the production of elemental iodine. Transport of shipments of iodide between Toronto Airport and Guelph during the times when the antibody binding was low, probably exposed the iodide to ambient temperatures of around freezing or below. However, this does not seem to provide the explanation for the seasonal variation. Notwithstanding the possible deterioration of the sodium iodide, the specific counts obtained per μg of iodinated pRXN were not different from those obtained during the warmer months. Moreover, the counting equipment that we use is housed in a centrally-heated, air conditioned facility and is used by several other research groups using a variety of different binding assays for a variety of substances. As none of them have reported any problems and because the specific counts that we obtained for pRXN did not differ significantly from season to season, it is difficult to believe that seasonally defective counting was the cause.

The coincidence of seasonal temperature change with loss of binding in our assay might be coincidental as factors other than temperature could be responsible. However, it is difficult to imagine what seasonal variable, other than temperature, could affect the assay so consistently.

Although at Bristol we were, and at Guelph probably still are, the most northerly laboratory in the world that operates a pRXN RIA, other laboratories, notably in Illinois and in Iowa, would experience winters of a comparable severity to those of southern Ontario.

We would be interested to learn if other laboratories that assay relaxin, or other peptides, have experienced similar problems.

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