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RADIOLABELLED D2 AGONISTS AS PROLACTINOMA IMAGING AGENTS

Progress Report

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Abstract

During the past year, further studies on mAChR were conducted. These studies included verification of the difference in pituitary distribution based on ligand charge. The pituitary localization of TRB, a neutral mAChR ligand, was verified. The lack of QNB blockade of TRB uptake was tested by blockade with scopolamine, another mAChR antagonist and by testing the effect in a different strain of rat. Neither scopolamine or change of rat strain had any effect. We concluded that TRB uptake in pituitary is not a receptor-mediated process. Further studies were conducted with an additional quaternized mAChR ligand: MQNB. Pituitary localization of MQNB, like MTRB, could be blocked by pretreatment with QNB. We have tentatively concluded that permanent charge on a mAChR antagonist changes the mechanism of uptake in the pituitary. Time course studies and the effects of DES on myocardial uptake are reported. A brief report on preliminary results of evaluation of quaternized mAChR ligands in the heart is included. In a limited series of such ligands, we have observed a single binding site and a difference in B_{max} values: QNB competition studies yield larger B_{max} values than studies with ^{3}H-NMS. Progress in the synthesis of D_2 agonists includes solving a synthetic problem and preparation of the "cold" analogue of N-0437 using procedures applicable to eventual synthesis with ^{11}C-CH_3I.

The grant year to be completed in January, 1990, has seen activity in two areas: description of the interaction of quaternized and neutral muscarinic receptor (mAChR) ligands with brain and peripheral tissues and synthetic efforts directed towards preparation of "cold" or standard compounds to be used for purposes of comparison and evaluation of D_2 ligands. Because the efforts are distinct, they will be summarized separately in the descriptions below.

Summary of Progress: Muscarinic Receptor Ligands

In the previous progress report, results comparing the potent, neutral mAChR antagonists, TRB, with the quaternized antagonist, MTRB, were reported. Of considerable interest, was the observation that TRB did not localize in the pituitary by a receptor-mediated mechanism. Pretreatment with QNB at a dose and time which reduced brain and heart radioactivity values by >90% had no effect on reducing radioactivity levels of ^{11}C-TRB in the pituitary. In contrast, the radioactivity levels in the pituitary of ^{11}C-MTRB could be reduced by about 70% with QNB pretreatment.

These results were sufficiently unexpected to require verification. In one test, the ability of scopolamine, another mAChR ligand, was used to block the receptors. The activity concentration of ^{11}C-TRB in control rats was 0.248 ± .070 %kg-dose/g and in scopolamine treated rats 0.183 ± .054 %, a non-significant difference. The results in pituitary tissue have generally been obtained in F344 rats, a strain genetically susceptible to prolactinoma induction by estradiol or diethylstilbestrol. To verify that the observed results were not dependent on rat strain, a similar study was conducted using Sprague-Dawley rats. The same general results were obtained: ^{11}C-TRB uptake in control pituitary was 1.68 ± 0.19 %dose/g and in QNB treated rats 1.86 ± 0.11. The absence of a receptor-mediated localization by neutral TRB appears to be confirmed.

The second phase of verification was to test whether similar results would be observed with other quaternized mAChR ligands. Thus, ^{11}C-MQNB (methyl quinuclidinyl benzilate) was prepared by Dr. GK Mulholland and its biodistribution was determined. A summary of those results is presented in Table 1. Data acquired using ^{11}C-MTRB and ^{11}C-TRB are also presented.

The expected difference in brain localization was observed: the neutral ligand had a three hundred fold higher brain concentration than did either of the permanently charged ligands. Heart localization was significantly different. The data were obtained at 30 min post injection. We have subsequently observed that MTRB behaves as an irreversible inhibitor at the myocardial mAChR but the MQNB has a rapid dissociation rate *in vivo*. The myocardial localization of MQNB and MTRB is a subject included in the competing renewal submitted. The pituitary uptake of all ligands was comparable.

Table 1. Comparison of ^{11}C-MTRB, ^{11}C-MQNB and ^{11}C-TRB in various rat[a] tissues at t = 30 min; data as %dose/g, N = 3.

Tissue	^{11}C-MTRB	^{11}C-MQNB	^{11}C-TRB
brain	0.009 ± .001	.010 ± .000	2.95 ± .17
pituitary	1.03 ± .12	.841 ± .138	1.24 ± .36
heart	11.75 ± .91	7.71 ± 1.54	4.95 ± .11
blood	.032 ± .005	.035 ± .014	.077 ± .004

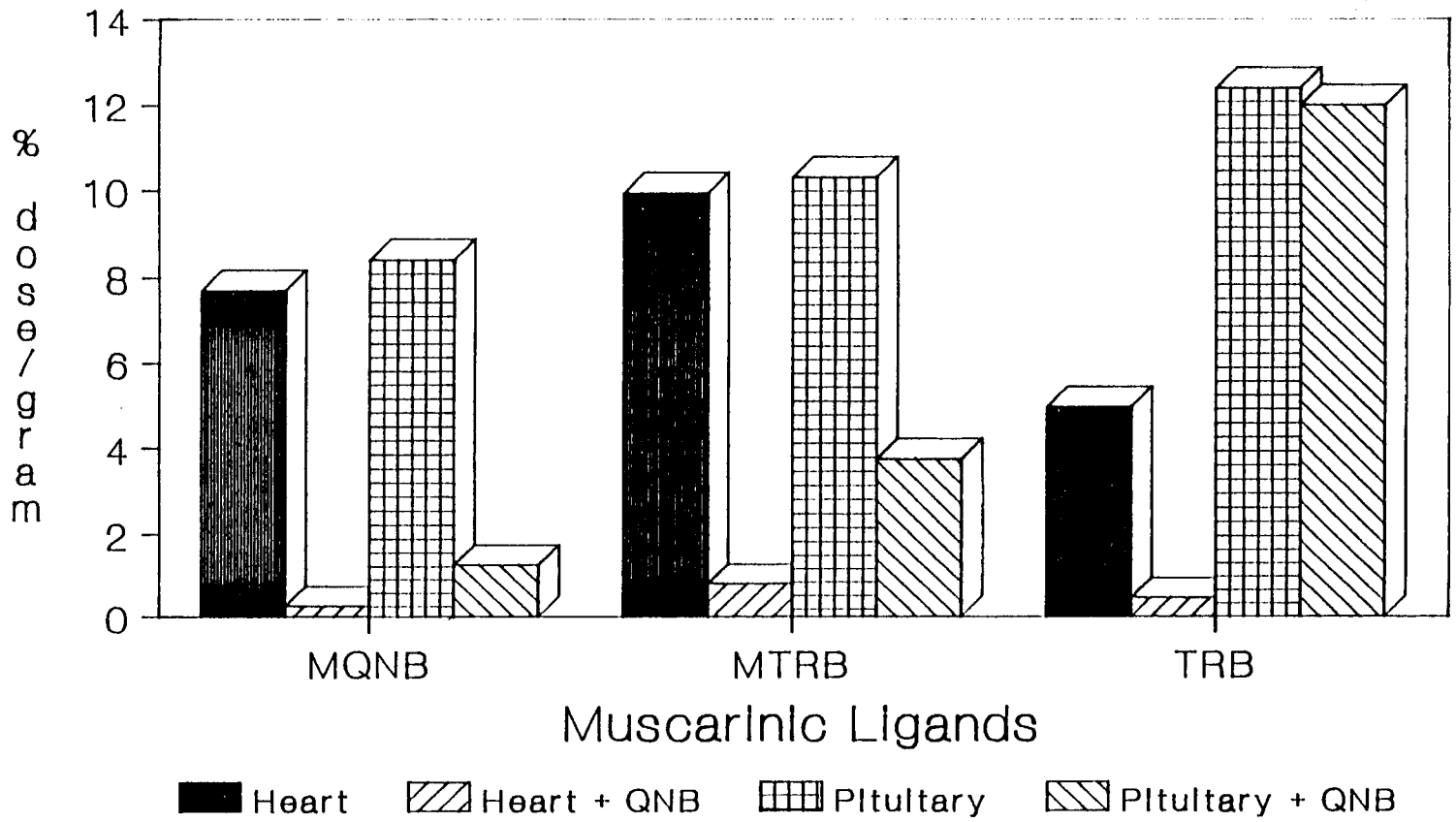
[a] Fischer F344 female rats.

Receptor-mediation of quaternized mAChR ligand localization in the pituitary was verified using ^{11}C-MQNB. Figure 2 shows the effects of QNB pretreatment on the tissue localization in the myocardium and in the pituitary. Results using ^{11}C-MTRB and ^{11}C-TRB are included for purposes of comparison. Based on the data we have obtained, there appears to be a distinct difference in pituitary localization which is dependent on the charge residing on nitrogen: neutral ligands localize but not by a receptor-mediated mechanism and charged ligands localize via a receptor-mediated mechanism.

To our knowledge there is only one other report describing the tissue localization of a muscarinic ligand in the pituitary. The mAChR ligand used was the neutral 4-N-methyl piperidyl benzilate (NMPB). The results obtained using TRB are not in agreement with results reported for NMPB (1). Under different experimental conditions, QNB pretreatment reduced uptake of NMPB by about 30% in the pituitary whereas the reduction was 74% in the medulla-pons. (Data in Figure 2 of Avissar, et al., was interpolated to obtain percentage QNB blocking values at t = 30 min.) The experimental conditions differed significantly in three areas: first, NMPB was injected subcutaneously whereas TRB was injected intravenously; second, the dose of QNB used in Avissar et al. was 3.3 mg/kg of active isomer whereas our dose was 1.0 mg/kg; and third, the time of pretreatment was 10 min vs. our time of 1 hr. The results reported for NMPB are questionable for several reasons: 1) the dose of QNB is sufficiently large enough to raise the issue of physiological effects such as blood flow changes, 2) the subcutaneous injection for both blocker and agent will increase any changes in distribution due to lipophilicity, and 3) the time between administration of blocker and agent may not be sufficient to permit adequate distribution of blocker. In order to determine if TRB and NMPB are indeed behaving differently at the pituitary mAChR or if experimental conditions are responsible for the discrepancy in results, several additional studies are planned.

We also compared the time course of ^{11}C-MTRB, ^{11}C-TRB and ^{11}C-NMPB in Sprague-Dawley rats. The time course differences are illustrated in Figure 3. TRB has higher localization values than either NMPB or MTRB, but, as discussed above, TRB apparently does not localize via a receptor-mediated mechanism. NMPB localization may not be receptor-

EFFECT OF QNB ON UPTAKE IN PITUITARY AND HEART



Pituitary values x 10

Figure 2

Time course of TRB, MTRB and NMPB

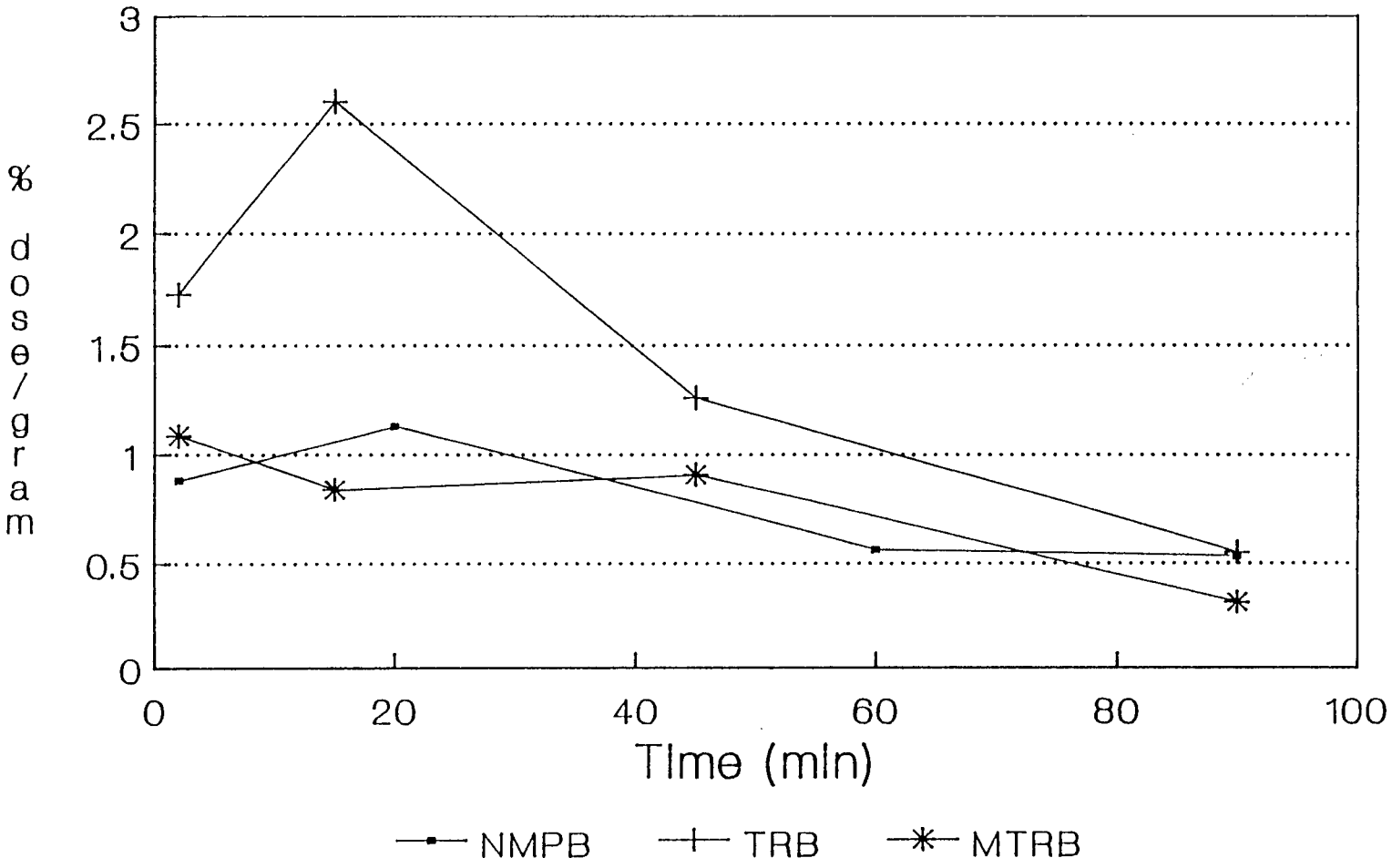


Figure 3

mediated - results from the studies of TRB using the conditions of Avissar are needed. Figure 4 shows that the time-activity curve for MTRB is similar in both Sprague-Dawley and F344 rats from 0 to 45 min although the activity values are higher in the F344 rat. The radioactivity concentration values in the DES-treated rat are not primarily a reflection of physiological differences between normal and abnormal pituitary tissue; they are, instead, largely a reflection of the difference in specific activities used (<200 Ci/mmol for the DES study and >1000 Ci/mmol for the F344 and Sprague-Dawley studies). The time course is quite different in the DES-treated rat: a 62% reduction was observed from 2 to 45 min whereas the activity appears to be constant in the normal rat over the same time frame.

Figure 5 illustrates the effect of DES exposure on uptake of MTRB in %dose/organ and %dose/g. As the length of DES exposure increases from 0 to 15 weeks, the %dose/organ increases by a factor of 28. However, the organ weight tissue increases by a factor of 36, resulting in a decreasing value for %dose/g. These data suggest that there may be some changes in either K_D or B_{max} of mAChR relative to normal values. The K_D and B_{max} values are in the process of being determined using both ^{3}H-QNB and ^{3}H-NMS. Similar data will be acquired in normal rat pituitary. Brain uptake (data not shown) is minimal as expected for a quaternized ligand. The heart uptake of MTRB appears to be constant from 6 to 15 weeks of exposure to DES which suggests minimal change in mAChR density or kinetic behavior occurs due to high circulating prolactin levels. The density of mAChR was reported to be constant in the myocardium of rats implanted with MtTW15 adenoma (Nelson, et al., 1987). We have determined K and B_{max} values in the myocardium and confirm Nelson's report of constant density of mAChR values in the heart.

The effects of permanent charge versus neutrality offer an additional mode for approaching imaging of other peripheral tissues. Pancreatic and intestinal localization of mAChR ligands may be influenced by ligand charge. The competing renewal for this grant presents proposed studies to explore this area more fully.

One of the studies proposed for this year (see last year's progress report) was completion of *in vitro* and *ex vivo* assays using quaternized mAChR ligands in the heart. The *in vitro* assays are only partially completed at this time. We have been conducting the assays in duplicate (at a minimum) and using both ^{3}H-QNB and ^{3}H-NMS as radioligands. The results so far with DAMP, N,N-dimethyl piperidyl benzilate, TRB and MTRB, indicate that one-site binding is observed for all agents. In general, the B_{max} values are lower for ^{3}H-NMS confirming results from chick hearts. Before moving into *ex vivo* assays, we wish to evaluate 3-fluoropropyl groups as quaternizing agents. The change in steric bulk between methyl and propyl groups may have a significant change on affinity. Further studies are detailed in the competing renewal.

Summary of Progress: D_2 Agonists

Progress has not been as rapid as hoped for. Synthesis of N-0437 analogues proceeded quickly and in excellent yield for each portion (i.e., synthesis of 5-methoxy-2-tetralone and synthesis of (2-aminoethyl)thiophene). However the reductive amination of the

Time Course of MTRB in Pituitary

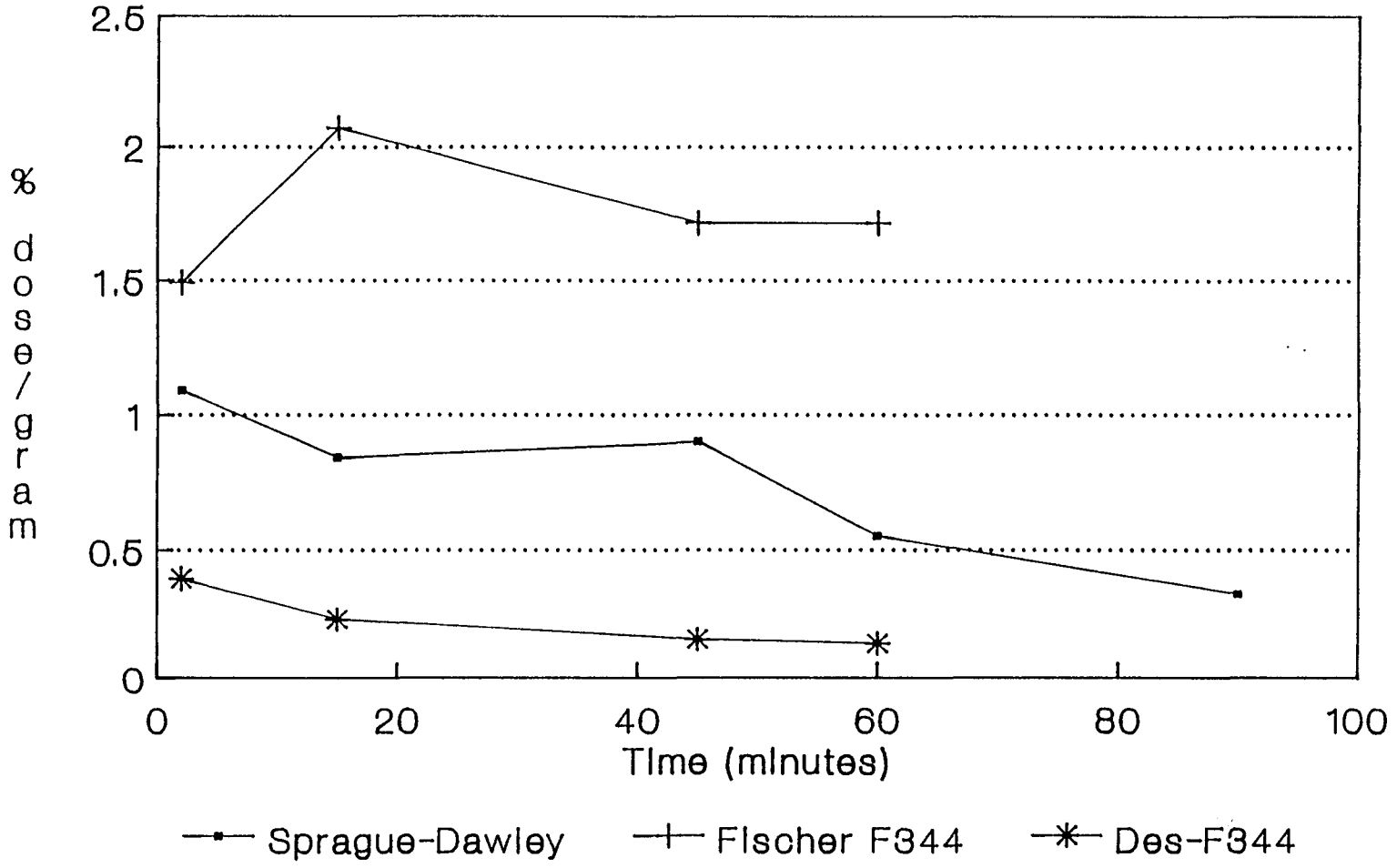


Figure 4

tetralone with the thiophene derivative yielded black, generally intractable tars. A number of synthetic variations were tried and none were successful. We have now discovered that a minor impurity carried along through recrystallizations and distillations of the tetralone is apparently responsible for the tars. The impurity has been removed and subsequent steps including the coupling of tetralone to thiophene derivative are now proceeding in excellent yields and we are obtaining white, crystalline products. The synthesis has been completed using a procedure directly applicable to ^{11}C synthesis. HPLC separation conditions for precursor and product are being developed.

The synthesis developed for N-0437 will yield a racemic mixture. Because of the distinctly different affinities frequently reported for pure enantiomers, we intend to prepare the pure enantiomers of N-0437; alternatively, we will attempt to resolve the racemic mixture using a chiral column. Further discussion is contained in the competing renewal.

References

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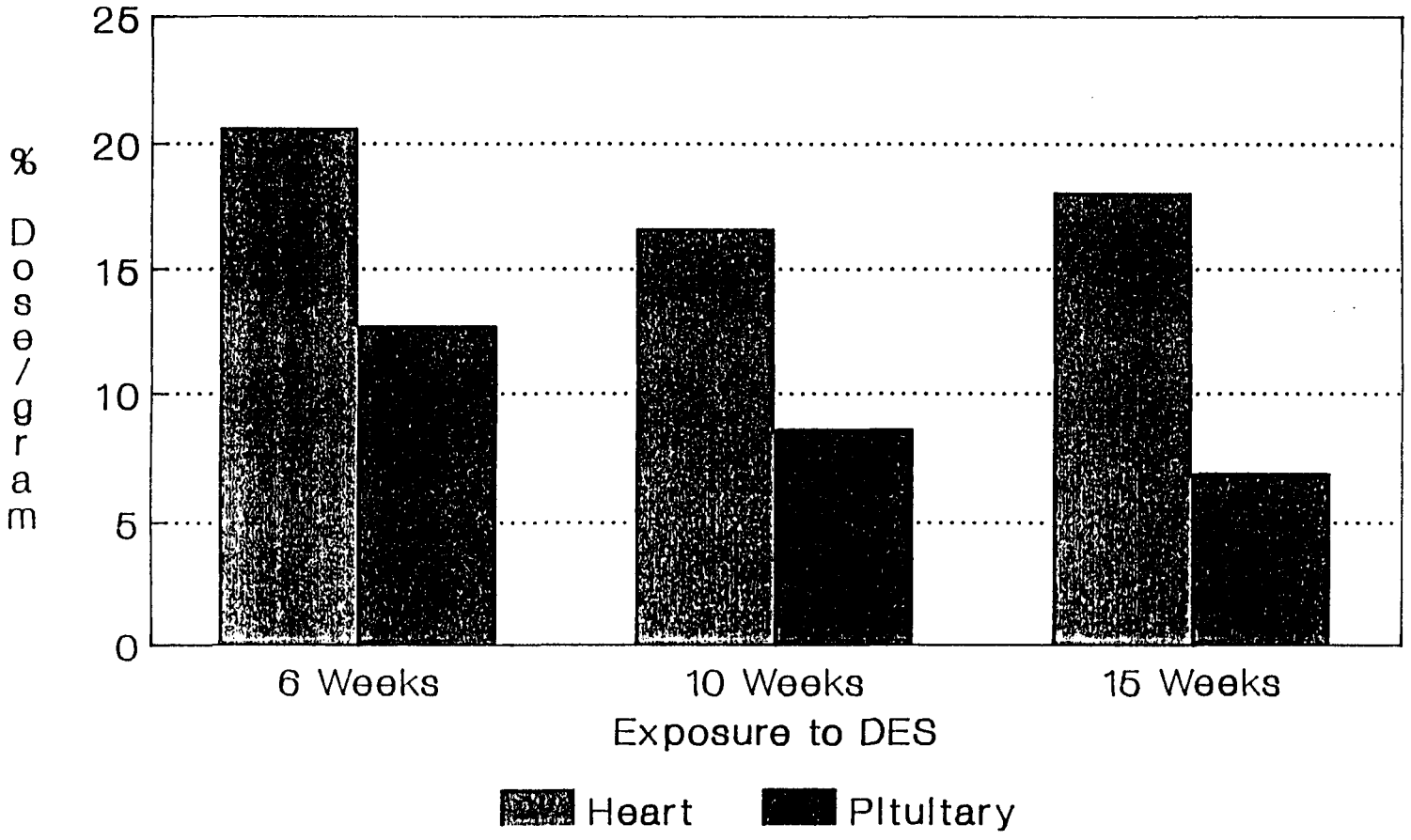
EFFECT OF DES EXPOSURE ON UPTAKE IN HEART AND PITUITARY



Pitultary values x 10

Figure 5