Radiopharmaceuticals for Receptor Imaging

There is a growing interest in the development of techniques for the external detection and quantification of receptor sites in man. The first step in this procedure is the development of radioligands, labeled at sufficiently high specific activities, which will bind to the receptor satisfactorily and provide a signal that can be measured externally. The synthesis of these compounds presents a significant challenge. In order to maximize the chances of success, the most obvious starting point for such work is to label a compound that has already been shown to localize on the receptor when labeled with tritium. Thus, the tritium-labeled compounds have become the "gold standard" against which all other compounds are measured. Nevertheless, this standard should always be treated with some caution as it can prove to be "iron pyrites" rather than "bankable bullion."

This point is elegantly demonstrated by the article in this issue on fluorine-18 haloperidol as a ligand for the murine neuroleptic receptor (*1*). Haloperidol and spiroperidol, drugs used for the control of schizophrenia, had both been shown to bind in vitro to the dopaminergic (neuroleptic) system, with spiroperidol having somewhat more favorable binding characteristics (*2*). When the compounds were tested in vivo, spiroperidol was, as expected, localized in the dopaminergic systems with striatum-to-cerebellum ratio of 5 to 1. Haloperidol however, was uniformly distributed throughout the brain (*3*). These data were clearly artifactual. If the theory as to the binding of these compounds were right, then the difference in the association constants of the two compounds as measured in vitro was not large enough to account for these differences in vivo. If the theory or the in vitro constants were wrong, then the distribution of either compound meant very little in terms of receptor binding. An indication of the nature of the problem is presented in the original article (*2*). The experimental section records that the assays with spiroperidol were straightforward but that considerable difficulty was experienced in washing off the free "haloperidol"—in fact, the free tritium.

Zanzonico et al. have shown that fluorine-18 haloperidol shows completely different distribution and kinetic data from that of the tritiated haloperidol and that the comparison of the fluorine-18 haloperidol with tritiated spiroperidol gives results in much better accord with the in vitro data. Re-examination of the tritiated haloperidol experiments shows that after an hour very little of the tritium is still associated with haloperidol, and that no tritiated compound can be identified, implying that the tritium has exchanged into water. This result was obtained with haloperidol tritiated in different positions. The rates of the process varied with the position of the tritium, but the final outcome was the same. When the same technique was applied to the fluorine-18 haloperidol, at least 80%, and probably 100%, of the fluorine-18 activity remains associated with the haloperidol peak on chromatography of tissue extracts. This finding shows not only that the carbon-fluorine bond remains intact while bound but also that the haloperidol itself is unchanged. Thus the tritium is being exchanged off the ligand rather than the ligand being metabolized. In the case of spiroperidol, the rate of exchange is much slower, but spiroperidol tritiated in different positions gives different results over time, suggesting that detritiation is occurring.

These results combine to show that when haloperidol retains its label in vivo, the distribution is broadly comparable to that shown by spiroperidol and that the more favorable brain uptake of haloperidol (~25%) compared with that of spiroperidol (1~2%) could make fluorine-18 haloperidol the radiopharmaceutical of choice for in vivo neuroleptic receptor measurements.

There are also some more disturbing implications in these results. When the concentrations of tritiated spiroperidol in the brain are followed over time, the expected increase in striatum-to-cerebellum ratio occurs as the specific sites slowly accept ligand from the nonspecific sites. After two hours however, the striatum-to-cerebellum ratio and the total brain tritium concentration falls. Fluorine-18 haloperidol does not show this decrease in total activity. Is the loss of tritium due to loss of spiroperidol or to tritium exchange? If it is due to tritium exchange, are the putative differ-

ent receptor populations due to real binding differences or to different rates of tritium exchange within the brain? In this context the tritium "gold standard" must be assayed by a more accurate technique than appearance.

In a wider context, several studies have shown that when standard procedures used for tritiated materials are transferred to radioligands of higher specific activity using different radionuclides, different data on receptor density are obtained (*4*). A recent publication has suggested that conventional procedures applied to the measurement of receptor density, although giving linear plots over the range studied, can rapidly become asymptotic on both axes and lead to errors in the estimates of both the receptor density and association constants (*5*). The tritiated ligands are normally used at specific activities of 10–30 curies per millimole, the highest values easily obtained. As a result, the quantitative in vivo data are obtained over a small portion of the semilogarithmic s-shaped binding curve, and the experimental requirements are such that these almost always lie on the same portion of the curve. Does this automatically give the correct answer or have the "constant" specific activity and the asymptotic errors combined to give a circular argument and a self-fulfilling prophecy concerning receptor density?

Furthermore, the combination of rapid association with the receptor, high first-pass extractions, and delivered doses well below saturation will lead to initial distributions of the radioligand that are heavily influenced by blood flow. The receptor must be present for specific uptake to occur, but the amount of ligand bound may be influenced by factors other than receptor density. Limited experiments have already shown that the percentage of brain uptake of haloperidol decreases as the total injected dose of haloperidol increases, suggesting that transport phenomena are important in localizing the ligands (*6*).

A chemically stable ligand containing a chemically stable radiolabel with a specific activity that can be usefully changed over several orders of magnitude without saturating the receptor is required to resolve all these questions. Such a ligand is necessary but will not in itself provide all the answers. It is only with such a ligand available, however, that the rest of the problem can be approached. The paper by Zanzonico et al. is a first step in this direction and we can expect more to follow.

TIMOTHY J. TEWSON
University of Texas Medical School at Houston
Houston, Texas

REFERENCES

1. ZANZONICO PB, BIGLER RE, SCHMALL B: Neuroleptic binding sites: specific labeling in mice with [^{18}F]-haloperidol, a potential tracer for positron-emission tomography. *J Nucl Med* 24:408–416, 1983

2. LEYSEN JE, GOMMEN W, LANDRON PM: Spiperone: A ligand of choice for neuroleptic receptors. I Kinetics and characteristics of in vitro binding. *Biochem Pharmacol* 27:307–316, 1978

3. NAYLOR RJ, OLLEY JE: The distribution of haloperidol in rat brain. *Br J Pharmacol* 36:208–209, 1979

4. KROHN KA, VERA DR, STADALNIK RC: A complementary Radiopharmaceutical and Mathematical Model for Quantitating Hepatic-Binding Protein Receptors. In *Receptor Binding Radiotracers Vol. II.* Eckelman, WC, Ed. CRC Press Boca Raton, Florida, 1982, 41–60

5. KLOTZ IM: Number of receptor sites from scatchard graphs: Facts and fantasies *Science* 217:1247–1249, 1982

6. TEWSON TJ, WELCH MJ, RAICHLE ME: Preliminary studies with ^{18}F-Haloperidol: A radioligand for in vivo studies of the dopamine receptor. *Brain Research* 192:291–298, 1980

Erratum

In the article entitled "[^{11}C] Spiroperidol: Synthesis, Specific Activity Determination, and Biodistribution in Mice," Volume 23, pp. 437–445, 1982, the following corrections should be noted:

page 439; Line 19: ppm should read ppb.
page 439, In "Synthesis of [^{11}C] Spiroperidol" line 6,
0.20 ml of 0.05 M NaOH should read 0.020 ml of 0.05 M NaOH