otherwise not be possible. Most receptors have very similar human and rodent sequences, but due to the small differences in primary amino acid sequence there have been cases of drugs developed for rats rather than for humans, because the compounds were active on the rat receptor but not on the human receptor.

It should be noted that the use of organ and whole animal pharmacology is still required. As previously noted, the cellular effect of receptor activation depends on the intracellular contents of the proteins involved in, e.g., the signaling cascades. These effects can only be determined when the receptor is situated in its natural environment rather than in a recombinant system. In most situations, both recombinant and in situ assays are thus used to fully evaluate the pharmacological profile of new ligands. Furthermore, once a compound with the desired selectivity profile has been identified in the recombinant assays, it is important to confirm that this compound has the predicted physiological effects in, e.g., primary nonrecombinant cell lines, isolated organs, and/or whole animals.

12.3.2 Binding versus Functional Assays

Binding assays used to be the method of choice for primary pharmacological evaluation, mainly due to the ease of these assays compared to functional assays which generally required more steps than binding assays. However, several factors have changed this perception: (1) biotechnological functional assays have evolved profoundly and have decreased the number of assay steps and increased the throughput dramatically, (2) functional assay equipment has been automated, (3) ligand binding requires a high-affinity ligand, which for many targets identified in genome projects simply does not exist, (4) binding assays are generally unable to discriminate between agonists and antagonists, (5) binding assays will generally only identify compounds binding to the same site as the radioactively labeled tracer. One important aspect of binding assays is the ability to determine ligand–receptor kinetics (on-rate, off-rate, and ligand residence time) which are important pharmacological properties affecting drug efficacy in vivo.

The Fluorometric Imaging Plate Reader (FLIPR™) illustrates this development toward functional assays. Cells transfected with a receptor coupled to increase in intracellular calcium levels (e.g., a $G\alpha_q$-coupled GPCR or a Ca^{2+} permeable ligand-gated ion channel) are loaded with the dye Fluo-3 which in itself is not fluorescent. However, as shown in Figure 12.11, the dye becomes fluorescent when exposed to Ca^{2+} in the cell in a concentration-dependent manner. In this manner, ligand concentration–response curves can be generated on the FLIPR very fast as it automatically reads all wells of a 96-, 384-, or 1534-well tissue culture plate. Many other functional assays along these lines have been developed in recent years. Importantly, the majority of these assays can be applied on both recombinant and native receptor expressing cell lines.

12.3.3 Partial and Full Agonists

Agonists are characterized by two pharmacological parameters: potency and maximal response. The most common way of describing the potency is by measurement of the agonist concentration which elicit 50% of the compound's own maximal response (the EC_{50} value). The maximal response is commonly described as percent of the maximal response of the endogenous agonist. The maximal response is also often described as efficacy or intrinsic activity which were defined by Stephenson and Ariëns, respectively. Compounds, such as 2-Me-5-HT and MK-212 in Figure 12.11, show a lower maximal response than the endogenous agonist and are termed partial agonists. The parameters potency and maximal response are independent of each other and on the same receptor it is thus possible to have, e.g., a highly potent partial agonist and a low potent full agonist. Both parameters are important for drug research, and it is thus desirable to have a pharmacological assay system which is able to determine both the potency and the maximal response of the tested ligands.