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General discussion

R. Leurs

We've heard interesting talks about new concepts like paradoxical pharmacology, endogenously appearing inverse agonists in the melanocortin field and intracellular proteins that might constrain the activity of some receptors. We had already discussed endogenously occurring inverse agonists, and someone mentioned that for the virally encoded receptors some chemokines might be recognised as such. I'm not sure if one of you would like to bring forward another example of evidence that it exists.

A. IJzerman

Indeed, it also struck me that the story on the endogenous inverse agonist has not advanced very much over the last 2 or 3 years, ever since that agouti-related protein was established as a potential inverse agonist. One could argue the human body has inverse agonists, the chemokines, to fight viral GPCRs. It is comparable to the GLP-1 receptor interacting with exendin, which is also an inverse endogenous agonist, but it stems from another species.

R. Bond

I don't like the idea of endogenous inverse agonists very much. Number one is that the kinetics are very slow. The body uses agonists versus agonists. It uses acetylcholine versus adrenaline because you can get millisecond regulation. So it doesn't appeal to me from a very fine-controlled type of thing to try and use an inverse agonist. The second thing is that I don't think we would see inverse agonism of drugs if we had to go through the whole worry of contamination with endogenous agonists. It wouldn't show up as readily as it does, if there were endogenous inverse agonists contaminating tissues. So I don't think we ought to stop, but I don't see it as a very likely scenario for a majority of neurotransmitters or anything like that.

R. Leurs

But how about receptors such as the 5-HT_{2C}, and histamine H_3, where there is really robust signalling?

R. Bond

Let us go back to the fact that there are nine official adrenoceptors subtypes. If adrenaline can't discriminate them, why are they there? It's not for drug companies to make money, it's for some other reason. And I think in part, it may set the background noise signal. In the heart, you want adenosine to have a break on it, don't waste neurotransmitter, put in a highly constitutively active receptor and you conserve a lot of energy. The β_2 has a lot more spontaneous activity than the β_1, so maybe the constitutive activity is to set

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baseline, to conserve neurotransmitter. Should that be down-regulated? Maybe, and you're dead right that where we start looking is the receptors that have high constitutive activity, but I don't think it's an a priori argument that we should have inverse agonists.

M. Brann

Yes, just to volunteer the reason why there's less progress is that who on earth's going to work on it? I think the analogous set of experiments is if you think about the orphan receptors. The orphans started getting cloned in 1986, and we have around 20 of them. Twenty of them, in 15 years, with massive effort, and who has done all of them? It's incredibly time consuming and expensive to do it. I mean nobody's doubting that those ligands exist and nobody's doubting that you know where to look for them.

T. Schwartz

It's a big challenge and it's not totally obvious. Take the MSH receptor, and then you've got agouti. You would tend to think it would be something looking like MSH, that should do it, and it's something totally different. In that respect, it may not be that simple. There are still many neuropeptides out there that do not have any function. The ones that had really high constitutive activity, which ones are there? I mean, there is a good reason for having a regulation of it. Otherwise, you could say why actually have—I mean, everything works fine with an agonist at different receptors. As soon as you've got this in here, you've got to have something regulating, it goes both up and down. If you only have a small amount of it, then there isn't really any big need to take it down. But it is very difficult to predict what it's got to be.

R. Leurs

What we learned is perhaps we don't have to look for ligands, but perhaps, we have to look for proteins. If you look at the Homer story for the glutamate receptor, there you have a protein.

T. Schwartz

Or look for co-expressed dimers. Just like when you're saying orphans—some of the orphans that are left may not at all have a ligand. I mean, they may be dimerisation partners, adhesion molecules and all kinds of things.

R. Leurs

Well that's a point, now you raise it: dimerisation in relation to constitutive activity. Are these two concepts related or not related at all? Who would like to comment on that?

A. Newman-Tancredi

5-HT_{1B} and 5-HT_{1D} receptors are examples where dimerisation occurs. These receptors are co-localised at a neuronal level and one of the things that we showed was that 5-HT_{1D} receptors are very highly constitutively active, whereas 5-HT_{1B} receptors are not. Susan George showed that these two receptors do dimerise. One can imagine that the 5-HT_{1D} receptor in a dimerised state with a 5-HT_{1B} may be modulating its constitutive activity. This, 5-HT_{1D} on its own, very high constitutive activity, 5-$HT_{1B\text{-}1D}$, D dimers, made with reduced constitutive activity. And these things are present on serotonergic cell bodies. I think there is a rationale, in certain cases, where you might want to go after that, and see if it really happens.

L. Prezeau

For the metabotropic glutamate receptors, there are functional dimers. You have several spliced variants for mGluR1. The long form, mGluR1a, interacts with Homer and it has a

very high constitutive activity. But the shorter forms, with a very short C-terminal tail, have no constitutive activity. Is there any cross-regulation of the constitutive activity? My conclusion is that the C-terminal tail is playing a very big job for that and the constitutive activity could be related to the dimerisation.

M. Brann

I've been actually quite fascinated by the way the field has evolved in the last 20 years. In the late 1980s, all of us looked very hard for dimers because you had the case of the tyrosine kinase receptors, the nuclear hormone receptors and so on. There were a lot of really powerful biological phenomena from dimers. Now, 20 years after, if you look at the literature with dimers, there's precious little in that field right now. And it's not because people haven't looked; people have been looking at this so intensively.

T. Schwartz

I admit I was an unbeliever. Now, to a certain degree—no, the point is that probably most of what we hear and see about ligand-induced dimerisation is probably not that at all. They are really dimers and there are some very good examples of it coming up now. We all want a reason on why should they dimerise? The good reason is that they have to be, like what has been shown for the $GABA_B$ receptor, they have to be two receptors and one G protein. That makes sense then. And all the things about oligomers and higher order things are probably related to aggregational receptors after stimulation. It appears very much that they're born as dimers, they live as dimers, but what sometimes is detectable is the confirmational change within a dimer. But there is no real ligand-induced dimerisation.

W. Clarke

I agree with Dr. Brann, in terms of the pharmacology about ligand binding. One would expect that there might be some form of cooperativity, i.e. when ligand A binds to one of the monomers that ought to influence the finding of ligand B—and whether ligand B is the same ligand or if it's a homo-dimer, or vice versa.

T. Schwartz

If you bind to one of them, the other one cannot bind. That is what is happening in the GABA receptor and in the mGluR.

R. Leurs

In another receptor, like the D_2 receptor, things might be different. Having the dimer where two linked ligands in fact were supposed to bind.

P. Strange

That's the model we put forward. We don't see much evidence in terms of dose–response curves, the Hill coefficient, and perhaps it is binding to one side of a dimer, when you stimulate with an agonist. But my feeling is that people have not looked very hard for dimers and haven't seen pharmacological evidence in ligand binding. But I'm not sure the work's been done, generally, carefully. My feeling is you have to do pretty detailed pharmacological studies to pick up some of these effects.

M. Brann

The kind of experiments that we did years ago was to do exactly the analogous experiments that the tyrosine kinase folks did, the whole idea of trying to go after dominant negatives, and the idea of titrating in and making hetero-dimers where you had a constitutively active one. None of those experiments worked.

P. Strange

Susan George has done that for the D_2 receptor showing a dominant negative effect.

A. IJzerman

Let us go back to inverse agonism again. Which structural organisation is needed to have inverse agonism? We've heard about the potential influence of oligodimerisation, we've heard about the Homer protein and the scaffolding system. What have we learned in the meantime about the cellular organisation with respect to the phenomenon of inverse agonism?

G. Milligan

One thing that's worried me a little bit today, although the story is obviously very clear, relates to the one that we just heard for Homer. It's almost as if in the native situation, having other protein scaffolds around, it is actually closing down and silencing even more than we might have expected in many receptors. My perception is that a lot of these receptor-interacting proteins are actually contributing to holding the receptor in an even more silent orientation than when we just do transfection studies in heterologous systems.

R. Leurs

What kind of proteins are you alluding to?

G. Milligan

Almost anything such as the sort of proteins that interact with the third intracellular loop of the dopamine D2 receptor. Almost every receptor now seems to have a set of interacting proteins. When you look at the β_2-adrenoceptor you can wonder how it ever moves around at all, there's so many potential partners for it.

R. Bond

That is my feeling. The receptor's natural conformation wants to be active, and we go out of our way to restrain it, to keep it inactive. But, it also gives a lot of things that can go wrong that will reveal constitutive activity.

G. Milligan

Though a lot of the old data, going right back to the early examples, that Mark Brann was mentioning would almost argue that the receptor is designed through evolution to try and stay quite inactive. If you remember Susanna Cotecchia's really classic paper on the α_{1b}-adrenoceptor, any of 20 mutations at the same position uncovered increasing degrees of constitutive activity. And the most silent one just happened to be the one that nature has come up with. Now, not many people have bothered doing all 20 mutants because it's a lot of work, but I think, conceptually, that it was such an important paper to me. About the fact that, really, you want agonists to signal, at least to that receptor, and presumably in many other receptors, yes, you want it fairly silent until the ligand comes along.

W. Clarke

Are you saying that you think inverse agonists are indeed pharmacological curiosities? Because if in vivo, the cellular systems are designed to keep constitutive activity down, with either Homer or other molecules, one then would predict that there would be very little in vivo effects of inverse agonism.

G. Milligan

To be honest, before today, I would have certainly been moving in that direction, it was something that we were all very excited about. I think the most exciting thing for me today

is seeing how many examples in a physiological setting people are starting to report. There was the one we heard about the α_1-adrenoceptors, or obviously, the mGluR system.

L. Prezeau

I would like to add just another comment on constitutive activity and pathology. In the same family as mGluRs and $GABA_B$ receptors, you have the calcium-sensing receptors that regulate the calcium level in blood, in fluids in the body, and you have a lot of mutants that give disease, like hypocalcaemia when the mutation results in constitutively active mutant. And you have mutations all over the receptors, from the C-terminal to the N-terminal tail. The receptor is something like 1000 residues long, and you have mutation everywhere. There are a lot of ways for getting the receptor active.

M. Brann

We published our experiments some years ago on the muscarinic receptor where we did random saturation mutagenesis. One of the most common phenotypes that you can get mutating the receptor is constitutive activity. You can get constitutive activity at bunches of residues in the cytoplasmic area, particularly on almost any place with a junction between the transmembrane domain, in extracellular or intracellular space. We interpreted that to mean that those residues are constraining the receptor and keeping it in an off state. The only explanation for how so many different changes could lead to the same — it seems like some kind of very specific structure of the inactive state- was it was being disrupted by all of those mutations. That's a very common phenotype, there's the example of the calcium receptor, there's the example of the V_2 vasopressin receptor, and there's the example of retinitis pigmentosa, all three of which where you have constitutively active mutations that can occur all through the receptor.

G. Milligan

I think that's also true, and maybe even more of an example than any of the rest has been the yeast pheromone receptor because it's been so easy there with the genetic readout to simply do complete random mutagenesis on the receptor. Virtually, everything activates it. So it is coming back to this issue that maybe we're trying very hard in nature to keep the receptor relatively silent. And maybe the ones we're all thinking about are the relatively unusual examples rather than the common ones.

L. Fagni

I would just like to stress a point that we also observed constitutive activity following some changes in sodium and potassium extracellular concentrations and also following the pre-activation of β-adrenoceptors. This may induce some drastic changes in the cell physiology. Maybe, it can induce some different types of changes in the interaction between the receptor and associated proteins, which can also induce physiological constitutive activity.

R. Leurs

I think you're right to mention that. The induction you have of your Homer1a protein, which in fact breaks down, this break on your glutamate receptor in epileptic conditions is a nice example how in pathophysiology, suddenly constitutive activity of these receptors might become more apparent than we see normally. Are there other examples that we know?

R. Bond

It certainly looks like in heart failure, the inverse agonists are working and the antagonists are not. I cannot think of a worse system in which to detect inverse agonism

than heart failure. The receptor has been chronically bombarded with agonist, desensitised, down-regulated, and isoprenaline can't give you a response. And yet the cell still knows the inverse agonist from the antagonist. I think whatever we use to detect inverse agonists we have to be very careful about because if it happens to be true in heart failure, that's probably the worst system that I can think of that would have the classically defined constitutively active receptor. And yet, it seems like the cell has no problem telling metoprolol and carvedilol apart from bucindolol.

W. Clarke

I think that brings up a real good point about cell state and dependence of inverse agonism in constitutive activity. Bouvier showed a few years ago that if you desensitised the β-adrenoceptor, dichloroisoproterenol (DCI, which was a partial agonist) became an inverse agonist in the receptors of the receptor population that remained responsive. We've seen similar data for the 5-HT_{2C} receptor system, that after desensitisation—even though that receptor's activity is particularly high—very low receptor expressions were able to see inverse agonist activity of drugs, after desensitisation. I think physiological changes inside a cell may bring—especially maybe in disease states—constitutive activity that wasn't there under normal circumstances.

T. Schwartz

That's a very interesting thing about that study because they detected that DCI was either an inverse agonist or a partial agonist, but it was never neutral. And they had a distribution of the efficacy that was going up and then going sharply down around zero and then up again. So, it shows it cannot really be a neutral antagonist.

One comment concerning the discussion on that all receptors were really kept totally shut or unshut, so there wasn't really any need for any inverse agonist for a while. Because we were talking about human receptors that really had pretty low starting point in constitutive activity. Is there any opinion about families of receptors that are really high in their constitutive activity? We just heard about some—the MC receptors, H receptors, and CB receptors and so forth—but there are many more from my point of view.

M. Brann

The H receptors are examples of the lipid-like guys in which we see blazing constitutive activity, but we can't really tell whether it's the ligand or the receptor.

P. Strange

I'll make some suggestions for the issues to consider during the discussion. So I thought that one of the things that has come out these days was the need to redefine mechanisms and models. During these days, we haven't really heard very much about allosteric effects except in relation to mGluRs, but there are allosteric effects on systems like muscarinic receptors, where there are allosteric inverse agonists, and I think, this is probably something that is worthwhile thinking about. And I also think the models really need to take account of trafficking. Organisation of receptors and its relation to the cytoskeleton, how does that affect inverse agonism? And dare I mention oligomers as well, that must be in there somewhere.

The topic that has exercised us nearly the most is defining the efficacy of ligands. How do we do it? I think that's really something that comes out of everything we have said here. Other issues: are the cell models that we are using adequate? Are they misleading us? Are the detection systems that we are using suitable? Is there a G protein

dependence for the structure—activity relationships? I think it might be useful to come back to that point in this discussion. Constitutive activity of receptors in vivo is very important also. We have heard some very convincing evidence that the receptors are constitutively active in vivo, and I think there's no question about that. What about the role of sensitisation, desensitisation, regulation of receptor constitutive activity, and the role of variants, the editing variants of the 5-HT_{2C} splice variants. We know there are diseases where there is constitutive activation, but how widespread is that? An important issue is the clinical relevance and what kind of compounds are we looking for in clinical applications. Do we want low efficacy compounds? Or do we want high efficacy compounds that are full inverse agonists, full agonists, and so on? What do we want? We haven't really said very much about acute versus chronic effects. To see an acute effect of an inverse agonist, you need to have substantial constitutive activation. I am not sure how to see a chronic effect. If you've got a good inverse agonist, it may do something to the receptor and change the receptor number in the absence of constitutive activation. How many natural inverse agonists are there? Should we look for those? Also, we have heard something about structure-activity relationships for inverse agonists. That seemed to me to be an important area to have a look at in the future. And it should be possible, eventually, to define the interactions of the compounds with the receptor. That must be another goal, but I share Newman-Tancredi's view that it's really difficult to do with the current level of knowledge. About pharmacogenomics, are there SNPs which affect inverse agonism? What do you think about all these ideas? In particular, it is very important to define efficacy. Should we simply be choosing a particular system to define efficacy? Should we be having multiple systems to define efficacy?

R. Adan

Maybe, what I am missing in the field a bit is the observation of real-time signalling of receptors. I had expected to hear a lot more about that, but why did I not? I can imagine that the use of FRET to monitor real time signalling, with fluorophores attached to receptors and Gα proteins would be helpful to investigate inverse agonist versus antagonist action.

T. Wurch

What we can do with the Ca^{2+} assays, in fact, is to study the antagonism in either antagonist-bound state or in an agonist-bound state, which is probably more relevant in the case of dopamine, because schizophrenia is a hyper-dopaminergic pathology, with increased brain dopamine levels. And under these conditions, by expressing a wild-type D_2 receptor and chimeric $G_{q/o}$ protein to couple the receptor to the Ca^{2+} response, we could differentiate between the response of the antagonists. But this differentiation is highly dependent on the stimulus given by dopamine. So for example, a compound which cannot inhibit this low magnitude phase at high dopamine concentration can totally reverse this response at the lower dopamine concentration. So this antagonist has to work probably with a tone of receptor activation which varies under pathological conditions, but also during the day, because they are produced with a kind of wave and so they are sometimes high, sometimes lower during the day, and so the antagonist that has to either block constitutive activation or just to block activation, so agonist-made activation has to play with all these different tones of the receptor.

P. Strange

I think it's a real goal to get a real-time readout. There is the G protein paper, isn't there? Do you know anything about that one, Milligan? The one with the alpha and beta subunits, with CFP, YFP, that's an example.

G. Milligan

There was obviously a FRET assay for G protein activation that's now available. It worked very well for the yeast system, however, people have not found it, unfortunately, easy to transfer the same constructs into the mammalian G proteins. And I think this is why we haven't really seen particularly fast advances there. Clearly, the other thing with trying to measure G protein activation in vivo, that has been very disappointing, and all the companies have worked on this for years, is trying to get a fluorescence-based assay for G protein activation. Unfortunately, the fluorescent labels that you are able to put into the backbone of GTP are just not bright enough to be able to see it in living cells, or even in membranes, so things like Mant-GTP are very effective if you use it on purified protein, but absolutely hopeless in a living cell, at this point. But I think things are coming. Obviously, we have been able to do single cell, real-time Ca^{2+} imaging for many years. Really, we are not far away now from seeing a whole slew of new reagents to be able to do this for c-AMP. The original one was a FRET-based assay from Tsien's laboratory, but it was far too complex to work in any reality. But I have seen two or three novel ones coming through now that should allow us to do single cell c-AMP imaging, and I think, we will see this during the next year as being a relatively straightforward reproducible and reliable system.

R. Bond

It's unfortunate that all the inverse agonists were once classified as antagonists. Because I see them as very active signalling molecules. And I think we tend to make the receptors that we're working on the centre of the universe, but when you go in and chronically administer a drug, in an integrated system with lots of compensatory responses, you are going to evoke signalling by chronically resetting the system. And I think, the inverse agonist also causes movement of the receptor, apart from other things. I'm not terribly concerned about whether an antagonist exists or not. But the other thing I did want to comment on was whether endogenous inverse agonists occur, and I still think that the answer to that is no. The reason is that the body invests a lot to amplify the agonist signalling, it's activation of currents, and calcium or phosphorylation. To have it shut off by an inverse agonist just seems like it would be a real big waste of energy, to have to shut all those mechanisms down again. So I don't think that the future is bright for endogenous inverse agonists.

T. Schwartz

Mark Brann was sort of turning things around, by saying it would be really interesting to have compounds that were neutral and had very little efficacy and so on. And also, it's sort of the same thing that Bond is now saying that basically all of them are antagonists at the same time. But there are systems where it probably will be possible to make them, because if you look at where small molecule compounds bind, if you're not a muscarinic receptor, then they are basically all binding in between the transmembrane helices and in some ways, we really can map them. And when you are down there, you're bound to shift the receptor, either towards one or the other state, they will bias the receptor for either

being in an inactive state or an active state. They will really do something to the receptor. Whereas probably some of the compounds that could be really interesting to do would be for some of the peptide receptors, where you can conceivably block the binding of the ligands, without actually affecting the state of the receptor very much. Of course, you have to do something like peptide analogues that will just disturb the binding, and thereby only disturb the binding of the endogenous ligands, and not actually shift the equilibrium. You can have binders that stick to extracellular domains that may not shift from one side to the other one. Going back to that experiment of Michel Bouvier, it always had efficacy going either left or right. It was never in the middle. And this tells us something very important, that if you're a small compound down between the transmembrane helices, there is no way you cannot do something, you are either shifting it to the right or the left. So perhaps to really have this, we should go and have something that binds to the extracellular part, and can disturb binding. Maybe on chemokines, we will have truly neutral ligands.

A. Newman-Tancredi

I'd like to come back on the issue of how to measure efficacy. One thing that has struck me during this meeting is the different models that have been developed to measure ligand efficacy, agonism or inverse agonism. And I think Giles mentioned this, commenting on how we needed more and more models to look at efficacy of drugs. Now, there's something that worries me a bit about that, it's that the more models you identify, generally what happens is you get further from the receptor. You look at downstream responses or different readouts, and the further you get away from the receptor, the more these responses are going to be cell-type-dependent, state-dependent. So answering the question on how to measure efficacy, ideally, we would be sticking as close to the receptor as possible, and therefore limiting the number of variables involved in the system, if we were really interested in looking at the receptor's constitutive activity *per se*, rather than some other intervening responses. And so my answer to that would be a change of receptor conformation, and I think this is where Lohse's data is particularly fascinating. Or something at the G protein, or very close to the receptor level. I don't know if other people would like to comment on that.

W. Clarke

I agree with you, what you're really looking for is intrinsic efficacy, which has been a difficult property to measure, and the notion that if we were able, in some magical way, to measure receptor conformations that can be elicited, or promoted or stabilised by ligands, and then developed numbers that relate to magnitude of those conformational changes. But I still think that the bottom line comes down to what do those conformations mean to a cell and to a system, and I think that a particular conformation can mean something different depending upon what signalling molecules may be available to that receptor, even though it is the same receptor conformation. If we just imagine three conformations and you imagine that one is completely inactive, it doesn't do anything, signalling molecules can't tell it's there. (I look up at the ceiling because sometimes I imagine myself inside the cell and there are receptors up there, and I'm trying to determine if there is one that's active, or another that is not active, or that there are two other conformations up there that are active.) Now, some molecules may be able to detect these different receptor conformations and some molecules won't. And if we go to cell type A versus cell type B, depending on what signalling molecules may be present they may or may not be able to see those

conformations. And then there are stoichiometry issues, how many signalling molecules present in different cells can lead to significantly different effects of the same receptor conformation. So the idea about intrinsic efficacy and being able to measure these conformations is, in some ways, academic. Ultimately, we need to know what happens in a cell or in the human body in response to ligand activation.

G. Milligan

I don't disagree with you there, but if we are going to take that rationally forward, then we should all give up using COS and HEK cells immediately. Because that's clearly not helping us in terms of the eventual endpoints that we really want to measure. We all do it for convenience, and I've got to say that I'd then agree with Newman-Tancredi that if you want to try and get measures that we can start to agree on round the table, then we have got to go for as high up the chain as possible. Because we are all going to use different systems, different expression levels, we are going to end up with different numbers that we are going to spend 3 or 4 days arguing about.

M. Brann

Part of this whole conversation is disturbingly reminiscent of the conversations that I was part of in the late 1970s and the early 1980s. It was the whole argument about the only way to do pharmacology is binding. Because if you do anything except binding, you're looking at things that really aren't on the receptor. And so, you have the school of how much of the exact ionic constraint do you do your binding assay in. And now we know, 25 years later that the absolute truth of pharmacology did not come from the fact that you measured the actual proteins. So, I think some of this is not so much about theory, and you're talking about theory, it's as much about practice. I think it's quite disturbing when you look at the history of the GTPγS binding experiments, and "oh, it's right next to the receptor!" And now, we see in these presentations of the last 3 days, sometimes it goes up, sometimes it goes down. And so in theory, it's right next to the receptor, but in practice, I've been hearing an awful lot of parameters, an awful lot of questions about exactly how you did it, and what did it mean and how did it mean that? So, is theory as important as practicality? I think pharmacology did a great job with reference compounds for 50 years, and if I had to go for it, I wouldn't worry so much about whether I measured GTPγS or measured "frog jumping rates". I'd want to measure things in the context of things I'd done before that have a relevance to something I can do a physiological measure with. And those are reference drugs, but pharmacology is pharmacology, and there are some principles that have really served us very well over many years, and I think we can't deny that there are antagonists and inverse agonists. Are people really seriously here debating that antagonists exist? I mean, they do exist, and this whole meeting's about a subclass of antagonists and trying to understand at a molecular level what antagonism is.

H. Giles

I would just pick up on what you're saying about looking back. And I think if you look back about how we classified agonists and how we measured intrinsic efficacy of agonists, it is in general true that if you choose to measure relative intrinsic efficacy, and I do go back to the relativity again, then it actually doesn't matter whether you are close to the receptor or far away from the receptor. You can still measure a relative number if you do your pharmacology right. And my gut feeling is that that's going to be the same for inverse agonism, it actually doesn't matter what your readout is, and I think that we need to start

generating some of those quantitative numbers whatever is our pet system that we are using, but generate those relativities. If we start seeing major differences and reversals of relative negative intrinsic activities, then we might need to question what are the right assays and what are the right numbers, whatever that might be. But until that point, I don't think we have enough information to say that there is a right assay or that it should be close to the receptor.

P. Strange

So do you think the problems are really going to occur only with the compounds near the margin? The arguments that the full agonists are always going to be full agonists and the full inverse agonists are always going to be full inverse agonists?

H. Giles

No, I don't. The point at where I struggle, in terms of the classification, is with these protean ligands, and I think some compounds are going to have more predisposition to exhibit a positive or a negative effect depending on the situation.

P. Strange

I guess there is something mysterious about those compounds that we really don't understand. Or is it that they just simply sit near the set point of the system, and if we use a different system with a different set point, they switch? That seems to me to be the nub of it.

T. Costa

I don't know what is more important for drug discovery, but I think that mechanisms and models are two different things. We are not going to learn things directly from models as they are only tools for trying to learn mechanisms. We need good biochemistry to learn mechanisms. One thing that I also find a bit disturbing is the confusion about the meaning of the states, R, R*, R**, etc. Those are not physical objects, those are functional states. We know almost nothing about their physics, but even if we did know, well... receptors are proteins, and so they do what the other proteins do, which is to explore an endless number of states. This is not surprising at all. Perhaps, the real question is: how come that with so many accessible physical states, these molecules are behaving like they existed in only one, two or three ? That's an interesting question. Another consideration is that no one has ever demonstrated that function can be associated with a particular physical state. It could just as well be the result of motion from one physical form to another. Thus, even if it was possible to identify all physical states that may not necessarily hack the mechanism. In conclusion, I think we should not take as physical objects the states that we actually measure in experiments; they are instead functional events. That helps, I think, in clarifying some confusion.