ABSTRACT

It is now well established that any given ligand for a G-protein-coupled receptor (GPCR) does not simply possess a single defined efficacy. Rather, a ligand possesses multiple efficacies, depending on the specific down-stream signal transduction pathway analyzed. This diversity may be based on ligand-specific GPCR conformations and is often referred to as “functional selectivity.” It has been known for a century that stereoisomers of catecholamines differ in their potency and, in some systems, also in their efficacy. However, the molecular basis for efficacy differences of GPCR ligand stereoisomers has remained poorly defined. In an elegant study published in this issue of Molecular Pharmacology, Woo et al. (p. 158) show that stereoisomers of the β2-adrenoceptor selective agonist fenoterol differentially activates Gs- and Gi-proteins in native rat cardiomyocytes. This study is so important because it is the first report to show that even the subtle structural differences within a ligand stereoisomer pair are sufficient to discriminate between GPCR conformations with distinct G-protein coupling properties. The study highlights of how important it is to examine the “more active” (eutomer) and the “less active” (distomer) stereoisomer to understand the mechanisms of action and the cellular effects of GPCR ligands. The study by Woo et al. will ignite a renaissance of the analysis of ligand stereoisomers, using sensitive pharmacological and biophysical assays. The available literature supports the notion that meticulous analysis of ligand stereoisomers is a goldmine for understanding mechanisms of GPCR activation, analysis of signal transduction pathways, development of new therapies for important diseases, and drug safety.
adds an additional layer of complexity to the topic of functional selectivity. Together with the light receptor, rhodopsin, the β2AR is the best-studied GPCR in terms of functional domains, ligand-receptor interactions, G-protein and effector coupling, physiological function (Gether and Kobilka, 1998; Rohrer and Kobilka, 1998), and high-resolution crystal structures (Cherezov et al., 2007; Rosenbaum et al., 2007). The catecholamines (−)-epinephrine, a hormone produced by the adrenal medulla, and (−)-norepinephrine, a neurotransmitter, are the endogenous ligands of the β2AR. The β2AR is a classic Gs-coupled GPCR, triggering activation of adenyl cyclase with subsequent cAMP production. In 1995, Xiao et al. made the very intriguing observation that pertussis toxin, ADP-ribosylating G-protein α-subunits and, therefore, uncoupling GPCRs from Gs, enhanced β2AR-mediated positive inotropic effects in isolated rat cardiomyocytes. These data suggested that in addition to Gs, the β2AR also couples to Gt-proteins in native systems. Then, in 2003, Xiao et al. noted that the positive inotropic responses toward most β2AR agonists such as zinterol, salbutamol (albuterol), and procaterol were enhanced by pertussis toxin pretreatment, whereas the effects of fenoterol were not affected. These findings were the first indication that fenoterol may stabilize a unique β2AR conformation promoting a distinct pattern of G-protein activation.

**Different Efficacies of GPCR Ligand Stereoisomers**

It has been known for a century that the naturally occurring (−)-stereoisomer of epinephrine constricts blood vessels more potently than (+)-epinephrine (Cushny, 1908). Since then, a large number of studies has elaborated the concept that (−)-stereoisomers of catecholamines bind to adrenergic receptors with higher affinity and activate the receptors with higher potency than the corresponding (+)-stereoisomers. This concept has recently been covered in an excellent review (Patil et al., 2008).

Intriguingly, some studies reported not only differences in affinity/potency between stereoisomers but also differences in efficacy. In particular, (−)-norepinephrine induces a slow conformational change in purified β2AR, whereas (+)-norepinephrine is inactive in this respect (Swaminath et al., 2004). In addition, in the presence of ITP or XTP, but not in the presence of GTP, (+)-isoproterenol is less efficacious than (−)-isoproterenol at activating adenyl cyclase by a β2AR-Gαs fusion protein (Seifert et al., 1999). There are also striking differences in the efficacies of (−)- and (+)-isoproterenol at stabilizing high-affinity agonist binding (i.e., ternary complex formation) in a constitutively activated β2AR mutant (β2AR CAM) (Seifert et al., 2001). Moreover, differences in efficacy between (−)- and (+)-epinephrine were observed in native tissues and cell membranes (Kim et al., 1981; Patil et al., 1996). Beyond catecholamines, dexmedetomidine is a partial α2A-adrenergic receptor agonist, whereas the other stereoisomer, levomedetomidine, acts as inverse agonist [i.e., a ligand that stabilizes the inactive (R) state of the GPCR] (Jansson et al., 1998). Furthermore, stereoisomers of chiraprofens, a new class of potent synthetic histamine H1-receptor agonists, exhibit striking species-specific pharmacological properties at the human, rat, guinea pig, and bovine H1-receptor (Strasser et al., 2008). Together, these data provide the basis for the concept that stereoisomers of a given ligand stabilize functionally distinct GPCR conformations.

**Differential Gs- and Gi-Protein Activation by Fenoterol Stereoisomers**

The analysis of the unique effects of fenoterol in cardiomyocytes was complicated by the fact that the compound possesses two chirality centers, yielding four fenoterol stereoisomers, referred to as (R,R)-, (R,S)-, (S,R)-, and (S,S)-fenoterol (Zojwia et al., 2007). The study of Woo et al. (2009) focused on the analysis of (R,R)- and (S,R)-fenoterol and the fenoterol derivatives (R,R)-methoxyfenoterol and (S,R)-methoxyfenoterol in isolated rat cardiomyocytes. The compounds studied exhibit selectivity for the β2AR relative to the β1AR, and the R,R stereoisomers possess considerably higher affinity for the β2AR than the S,R stereoisomers (Zojwia et al., 2007). (R,R)- and (S,R)-fenoterol are similarly efficacious at increasing contractility of the cardiomyocytes via the β2AR. Most strikingly, pertussis toxin shifts the contraction concentration-response curve for (S,R)-fenoterol to the left, whereas the stimulatory effects of (R,R)-fenoterol are unaffected by the toxin. Moreover, (S,R)-fenoterol stimulates extracellular signal-regulated kinase phosphorylation in a pertussis toxin-sensitive fashion, whereas the stimulatory effects of the corresponding R,R stereoisomers are pertussis toxin-insensitive. These data indicate that (R,R)-fenoterol activates only Gs-proteins, whereas (S,R)-fenoterol activates both Gs and Gαs (Fig. 1). This interpretation is supported by G-protein photoaffinity-labeling experiments. In particular, (R,R)-fenoterol is more effective at stimulating the incorporation of [γ-32P]GTP azidoanilide into Gαs than (S,R)-fenoterol, whereas only (S,R)-fenoterol stimulates photoaffinity labeling of Gsα2. Gsα2 is the predominant pertussis toxin-sensitive G-protein in the heart. Thus, the previously noted PTX-insensitivity of the effects of racemic fenoterol in cardiomyocytes (Xiao et al., 2003) could be explained by the fact that the higher potency of (R,R)-fenoterol at the β2AR for Gs masked the effect of (S,R)-fenoterol, possessing a lower potency for Gαs and Gs (Fig. 1). These data show that it is sometimes misleading to use the evaluative terms eutomer and distomer (introduced by E. J. Ariëns for the more and
less potent stereoisomer, respectively), which are by definition based on a particular affinity and/or potency. However, the distomer may actually possess more interesting and important pharmacological properties than the eutomer (Fig. 1).

There are some intriguing pharmacological differences in the effects of the \((R,R)\)- and \((S,R)\)-fenoterol pair relative to the \((R,R)\)- and \((S,R)\)-methoxyfenoterol pair in cardiomyocytes. In particular, \((R,R)\)- and \((S,R)\)-methoxyfenoterol are similarly less efficient at activating \(G_i\) compared with \((R,R)\)-fenoterol and also do not significantly couple to \(G_s\). Moreover, \((R,R)\)- and \((S,R)\)-methoxyfenoterol are more efficacious at activating \(G_\alpha_\text{q}\) compared with the corresponding \((R,R)\)- and \((S,R)\)-fenoterol stereoisomers. Pertussis toxin only slightly shifts the contraction-concentration-response curve for \((R,R)\)-methoxyfenoterol to the left because coupling to \(G_i\) predominates coupling to \(G_s\). This is not the case for both \(S,R\) derivatives, which, therefore, show distinct pertussis toxin sensitivity of the contractility. Thus, ligand stereoisomers may possess complex structure/activity relationships with respect to efficacy of G-protein activation that cannot readily be deduced from routine receptor affinity measurements (Jozwiak et al., 2007).

Viewed as a whole, the major contribution of the work by Woo et al. (2009) is to demonstrate ligand stereoisomer-specific activation of various G-protein isoforms that most likely results from stereoisomer-specific GPCR conformations. It is important to note that Woo et al. (2009) conducted their studies with isolated rat cardiomyocytes (i.e., a cell system that can be considered physiologically highly relevant). In support of the concept developed in this study are previous data from our laboratory using a recombinant expression system. In particular, the potencies and efficacies of a series of “standard” experimental \(\beta_2\)AR (partial) agonists [(−)-isoproterenol, salbutamol, dobutamine, (−)-ephedrine, and dichloroisoproterenol in order of decreasing efficacy] at \(\beta_2\)AR-G\(\alpha_\text{q}\) fusion proteins are quite substantially different from the corresponding parameters at \(\beta_2\)AR-G\(\alpha_\text{q}\) fusion proteins as assessed in the \([35\text{S}]\)guanosine-5'-[γ-thio]triphosphate binding assay (Wenzel-Seifert and Seifert, 2000). However, in our previous study, we missed the unique opportunity to dissect differences in G-protein coupling of ligand stereoisomers because we deemed this possibility as too unlikely, trying to “focus” our study on the supposedly “important” ligands. This is an excellent example of how wrong a priori probability assumptions can delay scientific progress for several years.

How Does the \(\beta_2\)AR Activate \(G_i\) Proteins?

In addition to making an important contribution to our understanding of the molecular mechanisms of GPCR activation, the study of Woo et al. (2009) yielded an unexpected novel approach to address the controversial and very important issue of how \(\beta_2\)ARs activate \(G_i\)-proteins. Previous reconstitution studies with purified human \(\beta_2\)AR and turkey \(\beta_2\)AR showed that \(\beta_2\)ARs can activate \(G_i\) in terms of guanine nucleotide exchange to some extent without the apparent need for additional proteins and phosphorylation reactions (Asano et al., 1984; Cerione et al., 1985). In agreement with these data, the analysis of \(\beta_2\)AR coexpressed with \(G_\alpha_\text{q}\) and of a \(\beta_2\)AR-G\(\alpha_\text{q}\) fusion protein in SF9 insect cell membranes revealed that the \(\beta_2\)AR activates \(G_\alpha_\text{q}G_\alpha_2\) more sluggishly than classic \(G_i\)-coupled receptors such as the formyl peptide receptor (Wenzel-Seifert and Seifert, 2000). In contrast, an alternative hypothesis had proposed that \(\beta_2\)AR-G\(\alpha_i\)-coupling depends on protein kinase A-mediated \(\beta_2\)AR phosphorylation (Daaka et al., 1997; Zamah et al., 2002). However, analysis of a \(\beta_2\)AR mutant devoid of protein kinase A phosphorylation sites did not support the latter hypothesis (Friedman et al., 2002). In their present study, Woo et al. (2009) found that all four fenoterol stereoisomers are similarly efficacious at stimulating protein kinase A-mediated \(\beta_2\)AR phosphorylation. The similar effects of stereoisomers on phosphorylation are in marked contrast to the differential effects of stereoisomers on activation of \(G_\alpha_\text{q}\), \(G_\alpha_\text{q}\), and \(G_\alpha_\text{q}\) as assessed by photoaffinity labeling. In unpublished studies performed by one of us (R.S.), preincubation of \(\beta_2\)AR-G\(\alpha_\text{q}\)-expressing SF9 membranes with the catalytic subunit of protein kinase A, ATP, and Mg\(^{2+}\) had no effect on the efficiency of GPCR/G-protein coupling. In aggregate, the available data indicate that protein kinase A-mediated phosphorylation is not a condition sine qua non for \(\beta_2\)AR/G\(i\) coupling to occur.

Interaction of Fenoterol Stereoisomers with the \(\beta_2\)AR at the Molecular Level

Although the study by Woo et al. (2009) constitutes a major advance in the field of GPCR conformations, several important questions remain to be solved. Most importantly, how do the assumed \((R,R)\)- and \((S,R)\)-fenoterol-specific \(\beta_2\)AR conformations look like at the molecular level? The ultimate answer to this question will have to come from crystal structures of different states of the \(\beta_2\)AR bound to fenoterol stereoisomers. So far, however, only the crystal structure of the \(\beta_2\)AR bound to an inverse agonist, carazolol, could be resolved (Cherezov et al., 2007; Rosenbaum et al., 2007). Given the high conformational flexibility and instability of the \(\beta_2\)AR, it will be a formidable challenge to obtain high-resolution \(\beta_2\)AR crystals bound to fenoterol. Perhaps this goal can be accomplished by generating \(\beta_2\)AR-Go(peptide) crystals as has been very recently shown for rhodopsin (Scheerer et al., 2008). It is certainly feasible to obtain insights into the mechanism of action of fenoterol stereoisomers by studying all steps of the G-protein cycle with \(\beta_2\)AR-Go fusion proteins (Wenzel-Seifert and Seifert, 2000; Weitl and Seifert, 2008) at high sensitivity and to conduct fluorescence spectroscopy studies with purified \(\beta_2\)AR (Swaminath et al., 2004). In addition, molecular modeling of the \(\beta_2\)AR ligand binding site based on the available crystal structures is possible (Audet and Bouvier, 2008).

Although the high-resolution \(\beta_2\)AR crystal structure probably represents an inactive receptor state, docking of the fenoterol stereoisomers may provide suggestions about possible reasons for functional selectivity. Figure 2 shows the minimized complexes of the \(\beta_2\)AR with \((R,R)\)- and \((S,R)\)-fenoterol, respectively. Both isoforms are docked in a favorable extended conformation close to an energy minimum. Assuming that the general binding mode corresponds to that of carazolol and that, in particular, the projection of the \(N\)-isopropyl and the \(N\)-1-methyl-2-phenylethyl substituent is similar, the \(\beta_2\)AR selectivity of the fenoterol and methoxyfenoterol isomers may be based on an H bond of the oxygen in the para position with the imidazolyl-NH of His-93 in TM2 (\(\beta_2\)AR: Ile). However, the para-hydroxy group of the fenoterol isomers can form a second H bond with the backbone of
Cys-191 in E2. This may account for the higher potency of (R,R)-fenoterol compared with (R,R)-methoxyfenoterol. The p-hydroxyphenyl ring of both fenoterol isomers is perpendicularly arranged with respect to the indole moiety of Trp-109 (TM3). Back-folding of the aralkyl group onto Tyr-308 (TM7), such as that suggested from CoMFA results (Jozwiak et al., 2007) and modeling (Audet and Bouvier, 2008; for bucindolol), is not likely in the case of the fenoterol derivatives as a result of steric hindrance in the new β2AR structure. The models in Fig. 2 are also consistent with the stereoselectivity at the C1 atom of N-1-methyl-2-phenylethyl substituted derivatives (Jozwiak et al., 2007) because, in the eutomeric R configuration, the methyl group may form direct van der Waals contacts with the side chains of Trp-109 and Thr-110 in TM3.

In the case of (R,R)-fenoterol, the ethanolamine moiety is involved in a distinct network of hydrogen bonds and ionic interactions with the β2AR (Fig. 2A). The protonated amine forms a salt bridge with Asp-113 (TM3) and a charge-assisted H bond with the side chain oxygen of Asn-312 (TM7). The hydroxy group may interact with the β2AR via two H bonds, one as donor for Asp-113 (charge-assisted), and another one as acceptor for the side-chain amide of Asn-312. The phenylethyl moiety closely fits into a hydrophobic pocket consisting of Val-114 and Val-117 (TM5), Phe-193 (E2), and Trp-286, Phe-289, and Phe-290 (TM6). Rotameric changes of Trp-286 are believed to induce the "toggle switch" for receptor activation (Schwartz et al., 2006). The meta-hydroxy groups form H bonds with Ser-204 and Ser-207, respectively, in TM5. Both serines are critical for catecholamine binding and β2AR activation (Strader et al., 1989).

In summary, the model in Fig. 2A suggests a very tight binding of (R,R)-fenoterol to the β2AR which is in particular based on seven hydrogen bonds. (S,R)-fenoterol can principally bind in a similar mode and conformation (see Fig. 2B). However, the interactions with the β2AR are weaker compared with the R,R stereoisomer because the orientation of the OH group does not enable the H bonds with Asp-113 and the amide NH2 of Asn-312 (instead, an H bond with the side-chain oxygen of this asparagine is possible). In the disrotomer S configuration, the hydroxy group is projected onto the phenyl ring of Phe-289. One meta-OH substituent forms an H bond with Ser-203 in place of that of the R,R isomer with Ser-204. The minimization has shifted the phenethanolamine moiety of (S,R)-fenoterol by up to 1 Å compared with its position in the β2AR-(R,R)-fenoterol complex, indicating greater flexibility as a result of lacking interactions. This phenomenon should be further analyzed by molecular dynamics simulations. To suggest a completely different binding mode of both fenoterol isomers (e.g., a reverse fit of the phenyl moieties in the case of the S,R-derivative) would be rather bold, because the stereoselectivity of the second chiral center is generally the same, independent of the configuration of the OH group.

Taken together, the models indicate possible reasons for the functional selectivity of fenoterol and methoxyfenoterol stereoisomers. It seems that deviations from the "normal" predominant Gs coupling of the β2AR and more promiscuity with respect to different G protein species just occur if certain interactions cannot be formed. Then the ligand-receptor complex has more degrees of conformational freedom than in the case of a tightly bound structure [such as (R,R)-fenoterol]. The higher flexibility enables the generation of a greater number of alternative conformations from which some may indeed represent "ligand-specific active receptor states," interacting more or less selectively with Gαi, Gαs, or Gα5. That is, ligand-specific inactive GPCR conformations are not...
a necessary condition for promiscuous G protein coupling. This hypothesis must be further substantiated by molecular dynamics simulations and can be verified only by crystal structures of active GPCR states. A first milestone on this long way has been very recently set by the release of an opsin structure in its G-protein-interacting conformation (Scheerer et al., 2008), showing an outward tilt of TM6 and a pairing of TM5 and 6.

Some Future Studies

(R,R)-Fenoterol is a Gₛ-selective full β₂AR agonist, whereas (S,R)-fenoterol is a partial β₂AR agonist with respect to Gₛ-activation and a full agonist with respect to Gₛ,β₁,γ₂-activation (Fig. 1). It will be important to develop G₁-selective β₂AR agonists to learn more about the as-yet-elusive (patho-)physiological relevance of the β₂AR-activated Gₛ pathway in cardiomyocytes and other systems such as bronchial smooth muscle cells. This will not be trivial, because to this end, the potency of most β₂AR agonists for the Gₛ pathway is considerably lower than for the Gₛ,β₁,γ₂ pathway (Wenzel-Seifert and Seifert, 2000). Moreover, high potency of G₁-selective β₂AR agonists would be most welcome to avoid potential ligand interactions with the β₂AR and β₁AR. Future studies will also have to address the question of whether in addition to Gₛ and G₁, fenoterol stereoisomers differentially activate pertussis toxin-insensitive Gₛ,γ₂-proteins that mediate phospholipase C activation. The necessity for such studies comes from the finding that the stimulatory effects of the four fenoterol stereoisomers examined exhibit striking differential and partial pertussis toxin-insensitivity, which is not discussed in the article by Woo et al. (2009). Previous studies from our laboratory have shown that the pharmacological profile of the β₂AR coupled to Gₛ-γ₂-proteins differs from the profile of the Gₛ and G₁-coupled β₂AR, although Gₛ-coupling was rather poor in our hands (Wenzel-Seifert and Seifert, 2000). It will also be necessary to study the impact of G-protein β₂,γ₂-complexes on Gₛα-selectivity of fenoterol stereoisomers. In particular, the stereoisomers differ remarkably from each other in terms of Gₛ,α activation, but they are quite similar in terms of β₂AR phosphorylation. An explanation for this discrepancy could be the recruitment of different Gβ₂,γ₂-complexes by various stereoisomers, compensating for the differences observed with respect to Gₛ,α activation.

The current study by Woo et al. (2009) focuses on efficacies of fenoterol stereoisomers with regard to the various parameters. Although this is sufficient to support the main hypothesis of the article, future studies should also carefully examine agonist potencies, because it is very possible that a given ligand possesses not only multiple efficacies but also multiple affinities for the various receptor conformations, depending on the specific parameter analyzed. These studies will bring us a step closer to the goal of achieving ligand-specific activation of Gₛ-protein subtypes. Multiple parameter-dependent affinities/potencies of a given ligand for the β₂AR have been observed before (Seifert et al., 1999, 2001; Weitl and Seifert, 2008).

Elegant studies with opioid receptors have shown that various ligands differ from each other in their membrane and cell compartment trafficking (Hanyaloglu and von Zastrow, 2008). Thus, it is conceivable that fenoterol stereoisomers show differences in β₂AR trafficking as well. In particular, resistance of fenoterol/β₂AR complexes to desensitization and internalization may occur because long-term treatment with fenoterol in a heart failure model is not associated with a loss of efficacy (Ahmet et al., 2008). In this context, it will also be interesting to examine the effects of fenoterol stereoisomers on long-term effects such as gene expression using the microarray technique.

The study by Woo et al. (2009) has important implications for future agonist screening programs in the pharmaceutical industry. It is clear that any drug development program dealing with stereoisomers should not only search for “more active” high-potency eutomers but also for “less active” low-potency distomers (Fig. 1). Moreover, it is prudent to study at least two independent signals for each GPCR, preferably mediated by different G-proteins. And even for GPCRs that couple to only one cognate G-protein, it is advisable to determine several signals at various steps of the G-protein cycle because differences of ligand potencies and efficacies for the various parameters within the cycle can be substantial, even if endogenous agonists are considered (Seifert et al., 1999, 2001; Weitl and Seifert, 2008). On first glance, this may sound like bad news because initially, a drug development program will become more complicated, expensive, and time-consuming. On second glance, however, the return may be novel interesting drugs that would have been missed using standard approaches aimed only at high-potency ligands and measuring only one signal. We missed such a great opportunity in our laboratory several years ago (Wenzel-Seifert and Seifert, 2000).

Clinical Implications

The present study has important clinical implications. In particular, fenoterol is widely used for the treatment of acute asthma attacks, and uncritical use of racemic fenoterol is associated with increased mortality of patients with asthma (Jalba, 2008). Thus, clinical studies will have to answer the question of whether specific fenoterol stereoisomers, through their unique pattern of G-protein activation, possess clinically relevant differences in terms of therapeutic efficacy and toxicity. Along the same line, albuterol possesses a chirality center (Boulton and Fawcett, 2002; Broadley, 2006). However, to this end, it is unclear whether levobuteral exhibits any relevant clinical advantages compared with racemic albuterol. Although additional clinical studies on this topic are certainly warranted, it is also important to characterize levalbuterol and dexamethasone in detail at the molecular and cellular levels, because so far, most mechanistic studies have been performed only with racemic albuterol (Seifert et al., 1999, 2001; Wenzel-Seifert and Seifert, 2000). The reason for this situation is simply the limited stereoisomer availability to the pharmaceutical community.

Another potential application of β₂AR agonists is the treatment of heart failure as adjunct to β₁AR antagonists. In particular, in a rat model of dilated cardiomyopathy, long-term treatment with racemic fenoterol enhances the beneficial effect of β₂AR blockade with metoprolol (Ahmet et al., 2008). Again, it will be interesting to examine the effects of fenoterol stereoisomers in this model. The efficacy of the agonist in this long-term treatment setting points to the lack of relevant desensitization.

Finally, we have recently shown that the endogenous β₂AR agonists (−)-epinephrine and (−)-norepinephrine interact differently with the β₂AR and β₁AR coupled to either of the
two Gα splice variants (Weitl and Seifert, 2008). Both catecholamines are used clinically in life-threatening conditions such as cardiac arrest and septic shock. The careful analysis of the corresponding (+)-enantiomers of catecholamines may yield drugs with improved clinical properties relative to the naturally occurring (−)-enantiomers. This possibility has not yet been explored at all. In this context, it will also be very interesting and important to determine whether fenoterol stereoisomers interact differentially with the various human β2AR polymorphic isoforms (Brodde, 2008).

Conclusions

The current study by Woo et al. (2009) corroborates the concept of ligand-specific GPCR conformations, resulting in differential G-protein activation or, in more general terms, functional selectivity. Ligand stereoisomers are important experimental tools to examine mechanisms of GPCR activation and signal transduction pathways. The present study should encourage pharmacologists to systematically examine the “more active” eutomer and the “less active” distomer of a given ligand and not focus only on more readily available racemic ligands or “eutomers.” We anticipate that analysis of ligand stereoisomers with modern pharmacological and biochemical methods will be a success and yield important data, ultimately improving clinical drug therapy and reducing drug toxicity.

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