



# Amiodarone and its putative metabolites fail to activate wild type *hTAAR1*

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## ABSTRACT

The ability of amiodarone and its putative metabolites to activate unmodified human trace amine associated receptor **1** (*hTAAR1*) stably expressed in CHO cell lines was evaluated. Receptor activation was monitored by measuring the accumulation of cAMP, the putative *hTAAR1* native second messenger, or calcium mobilization in cells where the receptor was coupled to the promiscuous Gq,  $G_{\alpha 16}$ . Despite literature reports of activation of rodent TAAR1 by these agents, no response was seen in either cell line although robust activation was obtained with the endogenous ligand  $\beta$ -phenethylamine. These results indicate that TAAR1 activation by amiodarone and its analogs is species specific.

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**Introduction.** The discovery and characterization of several families and subfamilies of mammalian trace amine associated receptors (TAARs)<sup>1–3</sup> has prompted much speculation<sup>4–8</sup> along with investigations<sup>9–13</sup> regarding the role of these G protein-coupled receptors (GPCRs). One line of investigation has demonstrated that iodothyronamines are potent agonists at rodent TAAR1 and this has been linked with the pharmacological effects of exogenously administered thyroid hormone metabolites such as T<sub>1</sub>AM in rodents.<sup>9</sup> Based on the known cardiac effects of thyronamines and their structural similarities with the clinical antiarrhythmic agent amiodarone (**1**), it has been speculated that TAAR1 may be involved in the actions of amiodarone.<sup>14</sup> Evaluation of amiodarone (**1**) along with a series of known and putative amiodarone metabolism products (**2–9**) (Fig. 1) in rat, mouse and rat–human chimera TAAR1<sup>14</sup> showed some agonist activity in rodent TAAR1, but not in the rat–human chimera cell line. The authors suggested that although amiodarone failed to demonstrate any activity in *r-hTAAR1*, some of the amiodarones may be active in unmodified *hTAAR1*. Since we had stably expressed wild type *hTAAR1* in two CHO cell lines, and had developed a rapid assay for evaluation of agonist activity of *hTAAR1*,<sup>15</sup> we were well positioned to carry out such studies.

**Materials and methods.** The amiodarones **1–9** (in dimethylsulfoxide) were a gift from Professor Scanlan;  $\beta$ -Phenethylamine hydrochloride was purchased from Sigma Aldrich (St. Louis, MO).

***hTAAR1* activation assays.** Receptor activation was assayed in CHO cells stably expressing  $G_{\alpha 16}$  (RD-HGA16 cells; Molecular Devices Corporation, Sunnyvale, CA) and *hTAAR1* as well as in CHO-K1 cells (ATCC) stably expressing only the *hTAAR1*, as previously

described.<sup>15</sup> The ability of the test compounds to activate *hTAAR1* was assessed in the RD-HGA16-derived cell line using the Calcium 3 Assay Kit (Molecular Devices; Sunnyvale, CA) per manufacturer's specifications except that the Calcium 3 dye was used at 1/3 the suggested concentration. In the CHO-K1 cell line, stimulation of *hTAAR1*-coupled adenylyl cyclase activity (cAMP Direct Biotrak EIA Kit; Amersham Biosciences, Piscataway, NJ) was used to evaluate the compounds. In each assay, the test compounds were assayed in duplicate at 0.1, 1.0 and 10  $\mu$ M (in 100% DMSO). Data are reported as percent maximal stimulation by 1  $\mu$ M  $\beta$ -PEA. The data represent the mean  $\pm$  SE from three experiments.

**Results.** The amiodarone series consisted of the parent compound **1**, its *N*-nor (**4**) and *N,N*-dinor (**7**) analogs, the moniodo (**2**) and desiodo (**3**) analogs, the moniodo-*N*-nor (**5**) and *N,N*-dinor (**8**), and the desiodo-*N*-nor (**6**) and *N,N*-dinor (**9**) analogs. At concentrations of 0.1 and 1  $\mu$ M compounds **1–9** elicited small negative responses in the cAMP assay. At 10  $\mu$ M very small (<5% of maximal activation by 1  $\mu$ M  $\beta$ -phenethylamine,  $\beta$ -PEA) positive effects were seen for compounds **6**, **7**, and **8**. (Fig. 2). In the calcium mobilization

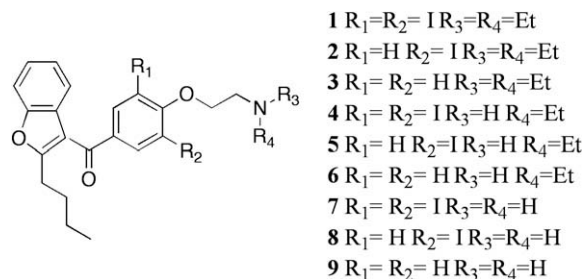
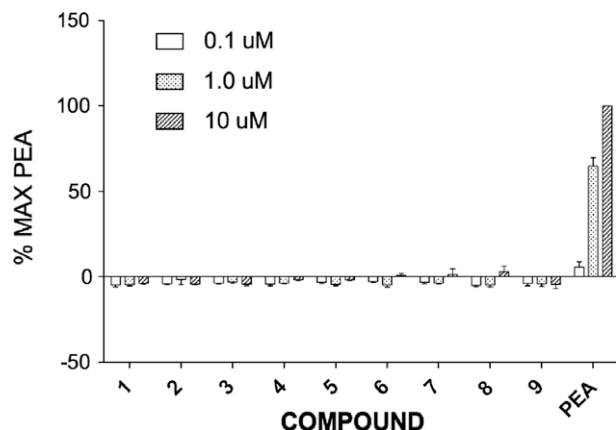


Figure 1. Structures of amiodarones **1–9**.

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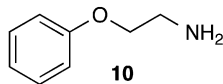


**Figure 2.** cAMP accumulation (% of maximal  $\beta$ -PEA response) resulting from interaction of compounds **1–9** with *hTAAR1* stably expressed in CHO-K1 cells.

assays amiodarone (**1**) failed to activate *hTAAR1* at any of the tested concentrations. The analogs **2–9** were inactive at 0.1 and 1  $\mu$ M concentrations (data not shown) but elicited calcium responses ranging in magnitude from 5% to 100% relative to  $\beta$ -PEA at 10  $\mu$ M. However, with the exception of compound **4**, similar responses (within the margin of the experimental error) were seen in the parent  $G_{\alpha 16}$ , non-*hTAAR1*-expressing, cell line. For *N*-noramiodarone (**4**) a net ( $RFU_{\text{expressing}} - RFU_{\text{parent}}$ ) stimulation of  $44 \pm 4\%$  relative to  $\beta$ -PEA was observed. Neither amiodarone (**1**), nor  $\beta$ -PEA, affected calcium mobilization in CHO cells stably expressing  $G_{\alpha 16}$  (no *hTAAR1*); a robust response was obtained for  $\beta$ -PEA in *hTAAR1*- $G_{\alpha 16}$  cells while **1**, amiodarone, failed to affect calcium flux in this cell line.

## Discussion

It had been suggested that some of the known side effects of amiodarone (**1**) may be associated with its, or its metabolism products', biological activity at TAAR1 and that the lack of any activity in the rat–human TAAR1 might be explained by the inherent difference between this chimeric *r-hTAAR1* and wild type *hTAAR1*.<sup>14</sup> Using our *hTAAR1*- $G_{\alpha 16}$  cell line we had determined that replacement of the ethylene group ( $CH_2CH_2$ ) in  $\beta$ -PEA by an oxyethylene group ( $OCH_2CH_2$ ) (to give **10**), reduced potency by an order of magnitude and reduced efficacy by a factor of two ( $EC_{50}$   $1,792 \pm 430$  nM;  $E_{\text{max}}$   $41 \pm 5$ , unpublished).



Moreover, our CoMFA of a series of systematically substituted  $\beta$ -PEA analogs, assayed using our *hTAAR1*- $G_{\alpha 16}$  cell line, had led to the conclusion that  $\beta$ -PEA analogs with bulky substituents are poorly accommodated by the receptor due to steric constraints, resulting in decreased potency and efficacy.<sup>16</sup> In particular, it appeared that *N,N*-dialkylation and *para*, or multiple, aryl substitutions resulted in a profound decrease in both potency and efficacy. In addition, it was observed that *ortho* substitution, particularly by halogens, enhanced potency. Based on these observations and conclusions, amiodarone (**1**) was not expected to be a potent agonist, but the *N,N*-dinor analogs **7** and **8** could have had some agonist activity. In fact, **7** and **8** had been found to be the most potent agonists in this series in the *rTAAR1* cell line, with **7** exhibiting full agonist activity at 10  $\mu$ M (relative to  $T_{1AM}$ ).<sup>14</sup>

Characterization of the action of the amiodarones on *hTAAR1* by measuring the accumulation of cAMP, the putative *hTAAR1* native

second messenger, in CHO cells stably expressing *hTAAR1* showed that none of the amiodarones **1–9** activated *hTAAR1* at 0.1–10  $\mu$ M (Fig. 2). This result was confirmed for 0.1–10  $\mu$ M concentrations of amiodarone (**1**) by examining the effect on calcium mobilization in CHO cells stably expressing *hTAAR1* coupled to the promiscuous  $G_q$ ,  $G_{\alpha 16}$  and for 0.1–1.0  $\mu$ M concentrations of the analogs **2–9**. At 10  $\mu$ M, compound **4** increased calcium mobilization by 44% relative to  $\beta$ -PEA, but the lack of a concentration response relationship does not support a *hTAAR1* mediated event. The reason for the responses observed for compounds **2–9** in CHO cells expressing  $G_{\alpha 16}$  but no *hTAAR1*, is not known. It should be noted that neither amiodarone (**1**), nor  $\beta$ -PEA, exhibited such behavior. These observations provide yet one more example of the lack of interspecies correspondence of TAAR1 responses.<sup>17</sup> Even in the rodent data it was noted<sup>14</sup> that while the desiodo analog **3** was a full agonist in *rTAAR1* at 10  $\mu$ M, it was totally inactive in *mTAAR1*. The reasons for these differences are not known. While both *mTAAR1* and *rTAAR1* consist of 999 base-pairs, several discrepancies have been noted for *rTAAR1*. By comparison, *hTAAR1* contains 1020 base-pairs with one discrepancy, possibly accounting for the difference in responses between rodent TAAR1 and *hTAAR1* to this class of compounds. Whether the accumulation of amiodarone (**1**), and/or of its possible metabolites **2–9**, in adipose tissue could lead to concentrations high enough (>10  $\mu$ M) to activate TAAR1 in humans, and thus be clinically relevant, as has been suggested,<sup>14</sup> is open to question. In rodents, the highest density of TAAR1 was found in the stomach, followed by the brain.<sup>1</sup> It would not be surprising to find significant differences in both the receptor distribution of TAAR1 and the metabolic fate of amiodarone between rodent and human, leaving the question of whether the side effects of amiodarone are mediated by *hTAAR1* unresolved.

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