European Journal of Pharmacology 587 (2008) 231–236

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Cardiac effects of trace amines: Pharmacological characterization of trace amine-associated receptors

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ARTICLE INFO

Article history:
Received 3 September 2007
Received in revised form 14 March 2008
Accepted 31 March 2008
Available online 7 April 2008

Keywords:
p-Tyramine
β-Phenylethylamine
Octopamine
Tryptamine
Thyronamine
Signal transduction
G-protein coupled receptor
Myocardial function

ABSTRACT

Trace amine-associated receptors, a novel class of G-protein coupled receptors which respond to trace amines but not to classical biogenic amines, have been found to be expressed in heart. Therefore, we investigated the cardiac effects of the trace amines *p*-tyramine, β-phenylethylamine, octopamine, and tryptamine. Isolated rat hearts were perfused in the presence of trace amines, monitoring the hemodynamic variables. In addition, radioligand binding experiments with [^{3}H]-*p*-tyramine and [^{125}I]-3-iodothyronamine were performed in rat ventricular tissue. Octopamine, β-phenylethylamine, and tryptamine produced a dose-dependent negative inotropic effect as shown by reduced cardiac output (IC_{50} = 109 μM, 159 μM, and 242 μM, respectively). In the same preparation a similar effect was produced by thyronamine and 3-iodothyronamine, with IC_{50} = 94 μM and 27 μM, respectively. The negative inotropic effect of octopamine was confirmed in a papillary muscle preparation. All trace amines except tryptamine increased the heart rate, but this action could be attributed to their sympathomimetic properties, since it was abolished by propranolol. The negative inotropic effect of trace amines was significantly increased by the tyrosine kinase inhibitor genistein. Specific and saturable binding of [^{3}H]-*p*-tyramine and [^{125}I]-3-iodothyronamine was observed in ventricular tissue. While [^{3}H]-*p*-tyramine was displaced by 3-iodothyronamine, [^{125}I]-3-iodothyronamine was not displaced by *p*-tyramine. In conclusion, trace amines and thyronamines are negative inotropic agents. Their effect appears to be mediated by a subtype of trace amine-associated receptor which is characterized by the rank of potency: 3-iodothyronamine > thyronamine = octopamine = β-phenylethylamine, while tryptamine and *p*-tyramine are significantly less active.

1. Introduction

We have recently reported that 3-iodotyhyronamine (T_1AM), a novel endogenous metabolite of thyroid hormone, produces negative inotropic and chronotropic actions in the isolated rat heart (Scanlan et al., 2004; Chiellini et al., 2007). T_1AM can activate trace amine-associated receptor 1 (TAAR1) (Scanlan et al., 2004), one member of a novel family of G-protein coupled receptors, whose name reflects the fact that it interacts with trace amines (i.e. *p*-tyramine, β-phenylethylamine, octopamine, and tryptamine), but not with classical biogenic amines.

After TAAR1 was identified (Borowsky et al., 2001; Bunzow et al., 2001) a comprehensive search for TAAR1-like sequences in several nucleotide sequence databases led to the identification of several additional mammalian TAAR genes (Lindemann et al., 2005; Zucchi et al., 2006; Grandy, 2007). Due to the lack of selective ligands or antibodies, subtype differentiation has been achieved on the basis of the pharmacological effects produced in cells expressing mRNAs that code for specific TAARs. Since we have recently reported at least 5 TAAR subtypes to be expressed in rat heart (Chiellini et al., 2007), in the present work we compare the response to T_1AM and to trace amines in order to obtain a pharmacological characterization of cardiac TAAR-mediated effects.

2. Materials and methods

2.1. Chemicals and radionuclides

T_1AM and thyronamine (T_0AM) were synthesized as described elsewhere (Hart et al., 2006). All other reagents were from Sigma-Aldrich (St. Louis, MO), or by Invitrogen Life Technologies (Paisley, UK). [^{3}H]-*p*-tyramine was obtained from Biotrend (Cologne, Germany),

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doi:10.1016/j.ejphar.2008.03.055

Table 1
Hemodynamic variables

Group	HR	AF	CF	CO	PAP
	%	%	%	%	%
Control	94±4	87±5	117±4	96±3	98±2
Genistein	104±1	69±4	123±11	87±4	83±7
Tyramine (100 μM)	133±6b	81±11	105±8	88±9	85±6a
Tyramine+genistein	114±4	27±13a	96±2	59±5a	81±3
β-Phenylethylamine (100 μM)	157±8b	55±16a	88±6a	66±12a	72±5b
β-Phenylethylamine+genistein	115±8	6±4b	90±3a	49±4b	85±5
Octopamine (100 μM)	134±9b	45±14b	75±22b	55±15b	74±5b
Octopamine+genistein	126±5	0±0 b	40±17b	26±2b	78±2
Tryptamine (100 μM)	120±7a	84±6	112±6	91±4	82±2a
Tryptamine+genistein	107±9	28±16a	100±6	71±5	94±3
Two-way ANOVA: effect of drug	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.05$
Two-way ANOVA: effect of genistein	$P<0.01$	$P<0.001$	NS	$P<0.001$	NS

Values are mean±S.E.M. of hemodynamic variables measured after 50 min of perfusion and expressed as percentage of the baseline values. Genistein concentration was 37 μM. HR, heart rate; AF, aortic flow; CF, coronary flow; CO, cardiac output; PAP, peak systolic aortic pressure. Results of two-way ANOVA are shown in the last two lines. a=$P<0.05$, b=$P<0.01$ vs the corresponding control group by Bonferroni posthoc test after two-way ANOVA.

[^{125}I]-T_1AM was synthesized as described elsewhere (Chiellini et al., 2007), using [^{125}I]-NaCl obtained from New England Nuclear (Milan, Italy).

2.2. Animals and perfusion technique

This investigation conforms to the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals. The project was approved by the Animal Care and Use committee of the University of Pisa. Male Wistar rats (275–300 g body weight), fed with standard diet, were anesthetized with a mixture of ether and air. After injection of 1000 U sodium heparin in the femoral vein, the heart was quickly excised and perfused according to the working heart technique, as described previously (Chiellini et al., 2007). The standard perfusion buffer included (mM): NaCl 118, $NaHCO_3$ 25, KCl 4.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 1.5, glucose 11. Perfusions were carried out using 200 ml of recirculating buffer, which was equilibrated with a mixture of O_2 (95%) and CO_2 (5%). Temperature was kept between 36.8 and 37 °C, and the pH was 7.4.

Unless otherwise specified, hearts were perfused for 50 min in the presence of specific trace amines or thyronamines, as detailed in the description of the individual experiments, and their hemodynamic variables, namely: aortic flow, coronary flow, cardiac output, aortic pressure, and heart rate were monitored. Powerlab/200 (ADInstruments, Castle Hill, Australia) was used for data acquisition.

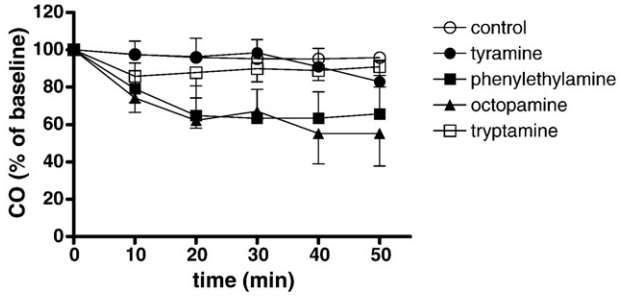

Fig. 1. Effects of trace amines on cardiac output. Hearts were perfused for 50 min with standard buffer, or with buffer containing *p*-tyramine, β-phenylethylamine, octopamine, or tryptamine at 100 μM concentration (n=4–7 per group). Data are shown as percentage of the baseline values. Actual baseline values are given in the text. Bars represent mean±S.E.M. (bars are not shown when smaller than symbol dimensions). Data analysis by two-way ANOVA yielded $P<0.001$ for the effect of treatment and $P<0.02$ for the effect of time. Bonferroni posthoc test for comparisons vs control yielded: $P<0.05$ at 20 min to 30 min and $P<0.01$ at 40 min to 50 min for β-phenylethylamine and octopamine; P = NS at all time points for *p*-tyramine and tryptamine.

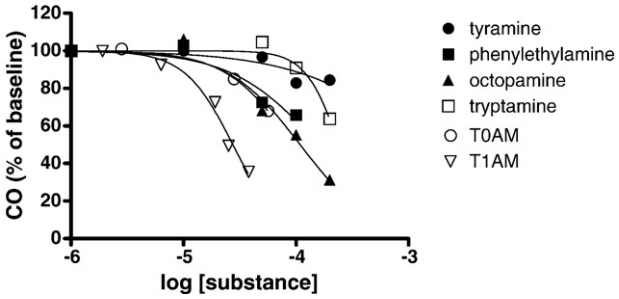

Fig. 2. Concentration–response curves for the effects of trace amines and thyronamines on cardiac output. Data points represents the values measured after 50 min of perfusion, expressed as percentage of the baseline values. Thyronamine data are derived from Chiellini et al. (2007). Bars represent mean±S.E.M. of 3–7 hearts per group. Results were analyzed by nonlinear regression using a sigmoid dose–response model to calculate the IC_{50}'s, which are shown in Table 2.

2.3. Papillary muscle experiments

The effect of octopamine was also investigated on isolated left ventricular papillary muscles (LVPMs) from five Wistar rats (260–285 g body weight). The animals were anesthetized by ether, and the heart was quickly removed. LVPM was carefully excised, clamped between two small metallic clips (Fine Science Tools, Vancouver, Canada) and vertically suspended in an organ bath (10 ml) containing pre-warmed (37 °C) Krebs–Henseleit solution with the following composition (in mM): NaCl 123, KCl 6.0, $CaCl_2$ 2.50, $MgSO_4$ 1.2, $NaHCO_3$ 20, KH_2PO_4 1.2 and glucose 11. The pH was maintained at 7.4 by bubbling with a 95% O_2–5% CO_2 gas mixture. The metallic clip on the upper end of the LVPM was attached to a force transducer (Mod. Wp1 Fort 10, 2200 μV V^{-1} g^{-1}, ADInstruments) and the other end was firmly fixed to the bottom of the organ bath. The muscle was electrically stimulated by means of two parallel platinum electrodes delivering 5 ms square pulses at 0.50 Hz and at current intensities (20–40 mA) which were 10% greater than the minimum necessary to produce a mechanical response. Transverse electrical field stimulation was supplied to the electrodes by high power constant current (Multiplexing pulser booster, Basile, Comerio, Italy). A Power Lab stimulator (ADInstruments) controlled the duration and frequency of electrical stimuli. After an equilibration period of about 1 h under a load of 1 g, the papillary muscle was gradually stretched to a length where the developed tension reached its maximal value (L_{max}). Experimental data were analyzed by a personal computer equipped with an analogical-to-digital converter program (PowerLab 400, ADInstruments). The action of cumulative doses (0.1 nM to 10 μM) of octopamine on LVPM contraction force was investigated at L_{max}.

Table 2
Negative inotropic effect of trace amines and thyronamines

	IC_{50} μM	R	P
3-Iodothyronamine (T_1AM)	>27	0.992	<0.01
Thyronamine (T_0AM)	>94	0.995	<0.01
Octopamine	>109	0.981	<0.01
β-Phenylethylamine	>159	0.954	<0.01
Tryptamine	>242	0.981	<0.01
Tyramine	>1000	–	–

IC_{50}'s for the effect of trace amines and thyronamines on cardiac output, calculated on the basis of nonlinear fitting of the experimental data shown in Fig. 2. Data for thyronamines are derived from Chiellini et al. (2007). IC_{50}'s are reported as > to the calculated values to remind that maximal effects were not actually determined. No IC_{50} is reported for tyramine because the effect was not statistically significant at any tested concentration.