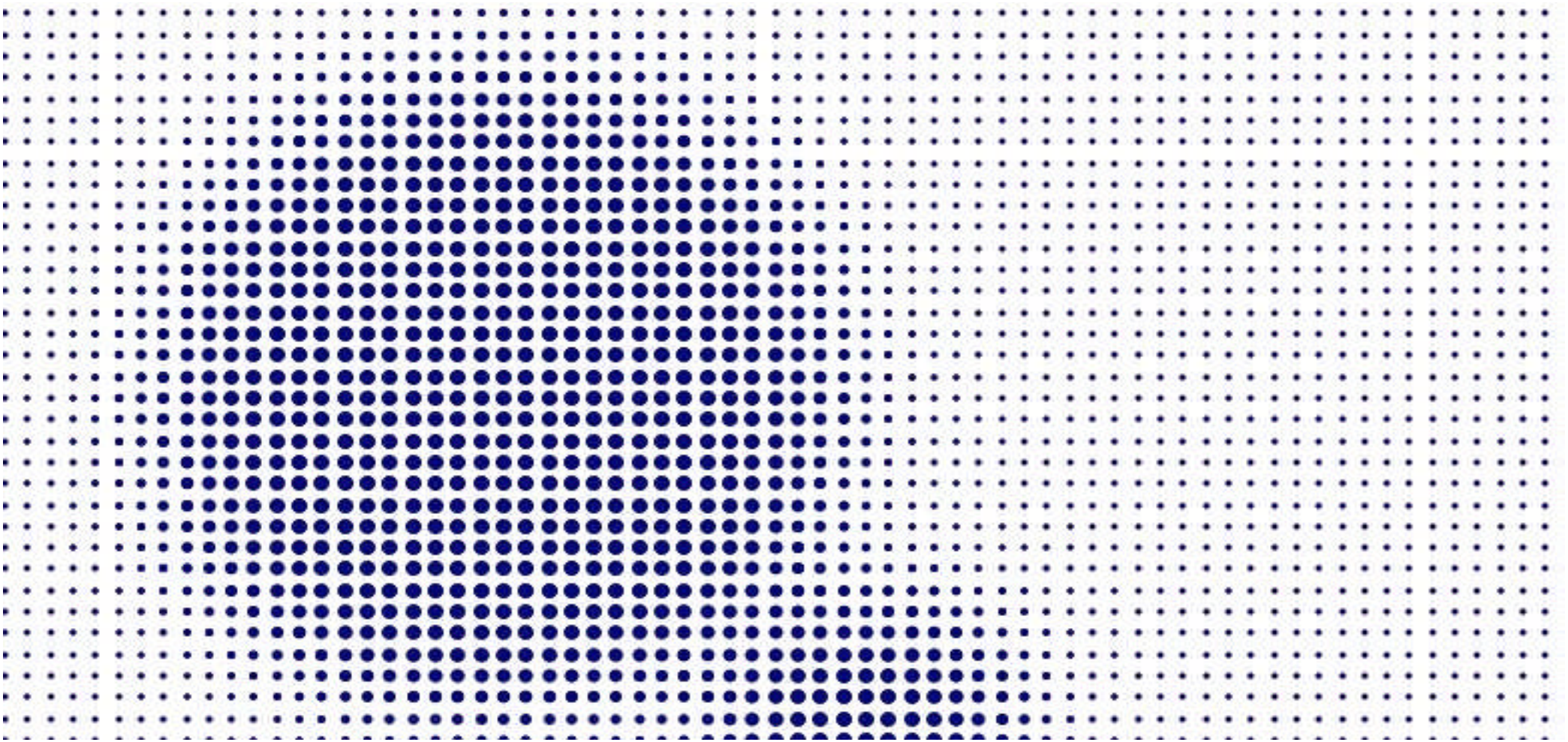
AGAH-Workshop “CHALLENGE AGENTS”
Hermann Fuder, Hamburg



Agenda Challenge Agents

- Definition
- Regulatory aspects
- Chemical Synthesis, Manufacturing & Quality
- IMP vs. Non-IMP
- Biological documentation
- Human experience
- Standardization

Types of Challenges in Early Clinical Development

- Pharmacological
- Toxicological (oxigen radicals)
- Functional/physiological (exercise/cold air)
- Behavioural/psychological

What are Challenge Agents?

- Agents which induce quantifiable physiological or pathological responses in healthy volunteers and patients in Phase I/II clinical research which allow the assessment of PD effects of NCEs in small cohorts of subjects under well-controlled conditions. The response to the challenge can be evaluated as a biomarker.
- Include agents ranging from approved prescription drugs, small molecules that elicit specific responses or, biological preps (e.g. allergen, attenuated virus).

Examples for Challenge Agents

Challenge agent	Activity	Route of adminstration and use
Allergens	Allergy	skin prick or inhalation (asthma only); to assess anti-allergy activity
AMP	Neurotransmitter/mediator release	inhalation; to assess anti-allergy effect
Capsaicin	Vanilloid receptor agonist	id, topical; pain model
CCK-4 (cholecystokinin)	**CCK receptor agonist**	**Induces panic attack**
Histamine	Histamine receptor agonist	Skin prick; to assess antiallergic effects
Hyoscine	Muscarine receptor antagonist	sc; dementia model
Ipecac	Inducer of nausea/vomiting	oral; to assess anti-emetic activity via 5-HT3 or NK1 receptor inhibition
Isoprenaline	ß-adrenoceptor agonist	iv; to assess blocking activity
Noradrenaline	Adrenoceptor agonist (α, ß)	iv; to assess blocking activity
Methacholine	**Muscarine receptor agonist**	**inhalation; to assess airway responsiveness**
Serotonin, Sumatriptan	5-HT agonist	iv; to assess blocking activity; GH response
Substance P	NK receptor agonist	skin prick; to assess NK blocking activity
Tyramine	**Noradrenaline release**	**oral or iv; to assess cheese effect/MAO-B selectivity**
CYP450 probes	Substrates for CYP450 subtypes	oral; to assess potential for interactions with established medicines

Purpose of Challenge Studies

- Proof of Principle/Concept (e.g. bronchoconstriction)
- Competitor/backup profiling
- Fulfill regulatory request (e.g. tyramine challenge)

Non-Investigational Medicinal Products (EUDRALEX Volume 10, Chapter V, 8 May 2007)

Non-Investigational Medicinal Products (= NIMPs)

- to induce a physiological or pharmacological response to assess the activities of an IMP (in which case they are often called **challenge agents**); or
- as support or escape medication for preventative, diagnostic or therapeutic reasons
- do not fall within the definition of an IMP.
- So, investigators who prepare a study with a non-IMP do not need an IMP.
- Nor does a trial of a non-IMP by itself require a CTA.

IMP vs. NIMP: Examples from MHRA Website

Example 6 Trial Design: Administration of capsaicin and morphine to healthy volunteers

- Purpose of Trial: A method validation study using capsaicin as a pharmacological agent to induce pain and morphine as a positive control
- Products Administered: Capsaicin as a solution for injection, Morphine sulphate as a solution for injection, Naloxone as a solution for injection
- Key Parameters: To determine the extent, reproducibility and reversibility of the pain produced, using morphine sulphate as a positive control
- Product Classification: Capsaicin NIMP, Morphine sulphate NIMP, Naloxone NIMP
- Rationale: This is a study to validate the pain inducing effect of capsaicin using a known analgesic as a positive control. The capsaicin is being used to induce pain by pharmacological means and is not an IMP. Since the morphine sulphate is being used as a positive control within the method validation, it is not regarded as an IMP for the purposes of the study. The naloxone is used to prevent unwanted opioid effects and is not an IMP. Since no investigational medicinal products are being administered, this is not a clinical trial within the scope of the Clinical Trials Directive

Challenge Agents are IMPs According to AMG-GCP-V

(3) **Prüfpräparate**

sind Darreichungsformen von Wirkstoffen oder Placebos, die in einer klinischen Prüfung am Menschen getestet oder als Vergleichspräparate verwendet oder

zum Erzeugen bestimmter Reaktionen am Menschen eingesetzt werden.

Hierzu gehören Arzneimittel, die nicht zugelassen sind, und zugelassene Arzneimittel, wenn diese im Rahmen einer klinischen Prüfung am Menschen in einer anderen als der zugelassenen Darreichungsform oder für ein nicht zugelassenes Anwendungsgebiet oder zum Erhalt zusätzlicher Informationen über das zugelassene Arzneimittel eingesetzt werden.

Requirements for Documentation of IMP

(4) Der zuständigen Bundesoberbehörde [**BfArM**] ist ferner vorzulegen:

1. das Dossier zum Prüfpräparat [**IMPD**] mit folgendem Inhalt:

a) **Unterlagen über Qualität und Herstellung [CM&C documentation],**

b) **Unterlagen über die pharmakologisch-toxikologischen Prüfungen [pharm.-tox. data]**

c) vorgesehene Kennzeichnung [labelling]

d) Herstellungserlaubnis [manufacturing license]

e) Einfuhrerlaubnis [import license]

f) **Unterlagen über Ergebnisse von bisher durchgeführten klinischen Prüfungen sowie weitere bekannt gewordene klinische Erkenntnisse [summary previous clinical experience]**

g) zusammenfassende Nutzen-Risiko-Bewertung [risk-benefit assessment]

GMP of Challenge Agent to be Confirmed by QP

The safeguarding of the clinical trial subject, in accordance with Article 3 and the objectives of the Directive is ensured inter alia by guaranteeing the quality and safety of the products and substances used in the trial. As NIMPs do not fall within the definition of investigational medicinal products, Articles 13 and 14 of Directive 2001/20/EC are not directly applicable. However, when NIMPs do not have a MA in the EEA, appropriate GMP requirements foreseen for the safety of the patients should still be applied and the sponsor should ensure that NIMPs are of appropriate quality for the purposes of the trial, taking into account, among other things, the source of the raw materials and any repackaging.

To meet the requirements of Articles 3(2) and as referred to in Article 6(3) of the Directive relating to protection of the trial subject, the same level of quality and safety should be ensured for the NIMPs as for the IMPs used in the trials.

Volume 10, **Clinical Trials,** Notice to Applicants,
Chapter V Additional Information

Classes of Challenge Agents

- **Class 1**: Agents that are approved medical products
- **Class 2**: Agents for which established clinical practice can be demonstrated but that are not approved medical products or that are approved medical products but that will be used in a manner not described by the label
- **Class 3**: Agents for which established medical practice cannot be demonstrated and that are not approved medical products
- Establish process within sponsor to endorse studies at different risk levels

Main Challenges in Preparing a Clinical Trial with a Challenge Agent

- Identify manufacturer/source of GMP NIMP material
- „IMPD“ (if needed in country) for NIMP („NIMPD“: quality and stability)
- Provide summary of pre-clinical and clinical experience with NIMP (White Paper)
- Standardization of challenge procedure
- Find experienced investigator/CRO
- Convince EC and RA
- All possible, but takes time, resources and needs effort

Manufacturing of Challenge Agents

- Manufacturers are „rare species“, many challenge agents **not** available as GMP products on the market
- Sponsor to manufacture challenge agent for own use
- Tedious expensive task to manufacture challenge agent taking up to a year
- Take into account in development plan early and initiate synthesis, formulation, quality and stability testing
- Prepare CM&C section of „IMPD“ to document GMP

Pharmacological and Toxicological Preclinical Data for Challenge Agent

- Use published sources
- Own preclinical/translational research (animal model similar to the human challenge, investigate influence of test IMP in animal model comparable to human model
- Include comparators in preclinical experiments
- Evaluate PK/PD relationships in animals

- **Limitation: toxicology data usually unavailable** and not requested for CTA

Summary of Experience with Use of Challenge Agent in Humans („White Paper“)

- Extensive review of published studies describing safety/tolerability, pharmacokinetics and pharmacodynamic effects in humans
- Prepare „White Paper“ matching section of IB describing human results
- Include extensive tabular work (and corresponding references)
  - justification of design/methodology
  - # of subjects in a given trial,
  - frequency and severity of AEs,
  - dose-response relationship,
  - interactions,
  - antidots and rescue medication,
  - risk management/emergency procedures

Standardization of Challenge Procedure

- Follow advise of scientific societies (e.g. bronchial challenge)
- Check regulatory standards or seek scientific advise from FDA or similar (e.g. tyramine challenge)
- New, previously unprecedented challenge type: validate model (reproducibility, sensitivity, etc.)

Validation of Challenge Model: Example of ATS Guidelines for Methacholine Challenge

Guidelines for Methacholine and Exercise Challenge Testing—1999

THIS OFFICIAL STATEMENT OF THE AMERICAN THORACIC SOCIETY WAS ADOPTED BY THE ATS BOARD OF DIRECTORS, JULY 1999

Am J Respir Crit Care Med Vol 161. pp 309–329, 2000
Internet address: www.atsjournals.org

Qualification of CRO to Perform Challenge Study

- Recent previous experience with challenge study of the planned type
- Proximity of intensive care unit
- Sponsor inspection/audit of site before commitment

Practical Example: Tyramine Challenge

- Physiology/pathophysiology
- Aim
- Regulatory standard: FDA advise

Sources of Tyramine

- Tyramine is formed by the degradation of protein in foods:

Protein → Tyrosine → Tyramine

- Thus, it is found in relatively large amounts in many foods that have undergone aging, such as many cheeses.

Tyramine Content in a High-Tyramine Meal

Sample Meal	Portion (g or mL)	Tyramine content (mg/portion)
Bottled beer	700	1.0
Sauerkraut	454	16.7
Boiled potatoes	454	1.9
Green and yellow peppers	250	1.8
Roast Pork	454	3.2
Pepper sauce	150	3.0
Cheese		
Camembert	30	1.4
Danish blue	30	8.8
Pinot Noir	700	2.0
Total tyramine in meal		39.8

Tyramine Pharmacology

- Structurally similar to adrenaline and noradrenaline but weaker action.
- Once systemically absorbed, it is taken up by adrenergic neurons and displaces noradrenaline from synaptic vesicles.
- Large amounts of noradrenaline are released: vasoconstriction and potentially hypertensive crisis, arrhythmias (bradycardia, tachycardia, ES).

Natural Protective Mechanism Against Excessive Tyramine

- Tyramine is metabolized by monoamine oxidase-type A (MAO-A)
- Pre-systemic (major role)
  - intestinal wall
  - liver (first-pass effect)
- Systemic (minor role: normally <1%)
  - peripheral adrenergic neurons

"Cheese Reaction" Frequently Seen With Old MAO Inhibitors

- large systolic blood pressure increase (mean increase of 55 mmHg) (SBP>DBP)
- increase in pulse, palpitations
- headache
- nausea or vomiting
- diaphoresis
- photophobia
- rare intracerebral bleed, cardiac failure, or death

Traditional Antidepressant MAOI's (e.g., Tranylcypromine)

- Inhibit MAO-A at clinical doses and allow large amounts of tyramine to enter systemic circulation → cheese reaction.
- Thus, foods and beverages with high tyramine content are prohibited.
- Also, inhibition is irreversible: enzyme "killers." After TX, MAO-A must be regenerated (2 wks).

Tyramine Challenge Studies Requested by RA

Objective

Provide a clinically relevant measure of the degree of MAO-A inhibition as reflected by the estimated minimum dose of tyramine that produces a clinically significant rise in blood pressure.

Lower minimum tyramine dose = greater inhibition.

Regulatory request for this study, no easy escape!

Challenge Study Characteristics

- small N (10-20 subjects).
- young to middle-age healthy volunteers.
- usually under fasted conditions.
- tyramine administered in capsules.
- few included a comparator group.
- Daily escalating/decreasing doses

Overall Study Design

Baseline tyramine challenge 1

↓

Baseline tyramine challenge 2

↓

Study drug treatment

↓

On-drug tyramine challenge

Outcome Variables of Interest

- Pressor Dose or TYR30 or PD30 = estimated minimum tyramine dose required to produce the BP endpoint (increase in systolic blood pressure by 30 mmHg) at each challenge.
- Baseline Pressor Dose = mean of the pressor doses for 2 baseline challenges.
- Tyramine Sensitivity Factor (TSF) = ratio of the baseline to the endpoint pressor dose.

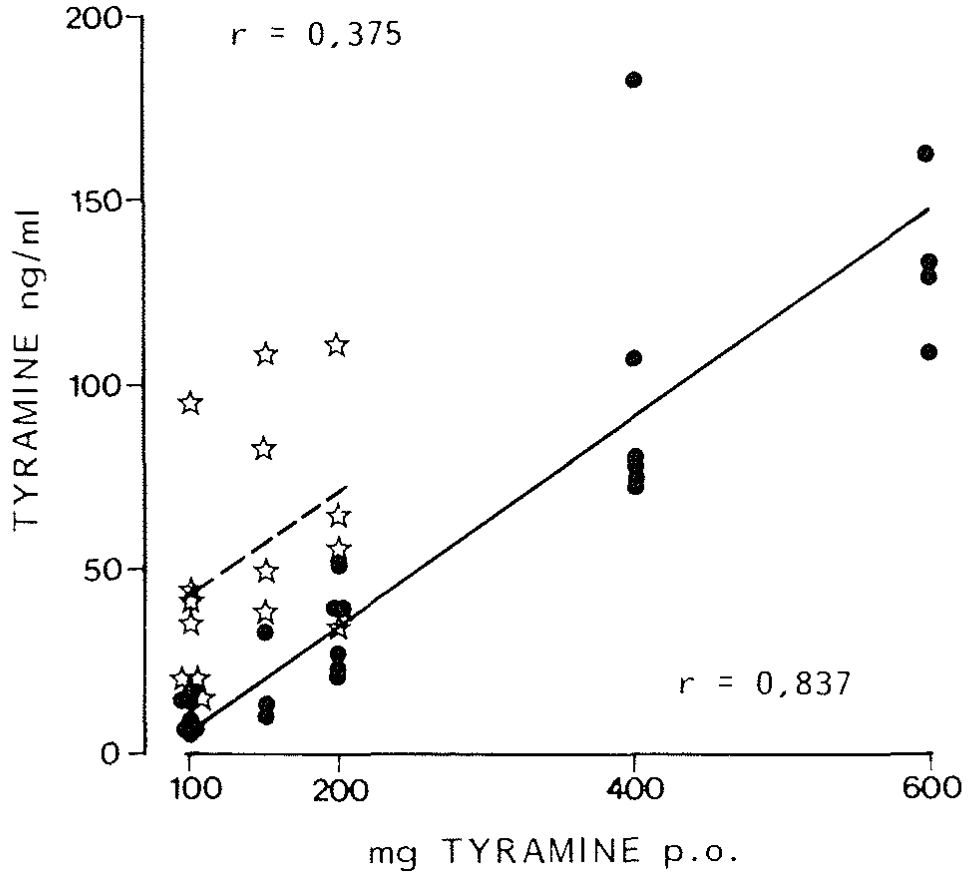
Type of MAO Inhibition and Tyramine Test: p.o. vs. i.v. Challenge

- Tyramine test p.o.: pressor response induced after oral tyramine dosing ("physiological" way of tyramine administration: red wine, cheese, pickles)
  - Detects MAO-A and –B inhibition
  - Reveals relevant interaction between food containing tyramine and drug
  - Large variability
  - Less acceptable to authorities
- Tyramine test i.v.: pressor response induced by "bolus" tyramine injection
  - Detects MAO-B (and –A) inhibition
  - Low variability
  - Risk of hypertensive crisis in in-experienced hands (antidote: i.v. phentolamine)
  - Acceptable to authorities to avoid label of risk of tyramine interaction
- To be discussed: single dose vs. multiple dose design with test drug

Regulatory Guidance for Challenge Study: Example for Criteria of FDA

- Adequate sample size, male and female subjects
- Positive control to be included
- Adequately bioavailable tyramine product
- Tyramine to be administered p.o. in fasted state
- Tyramine dose-response curve (e.g. from 25 mg to up to 800 mg, doses to be administered on subsequent days), not just single dose of tyramine
- Criterion PD30: increase in systolic blood pressure by > 30 mmHg (in at least 3 subsequent measurements at defined intervals e.g. 3 or 5 min)
- Several, adequate dose levels of test drug including a supratherapeutical one, under steady state (for each a tyramine dose-response curve needed)
- Plasma tyramine

Example Tyramine Effect (Korn et al. 1988)



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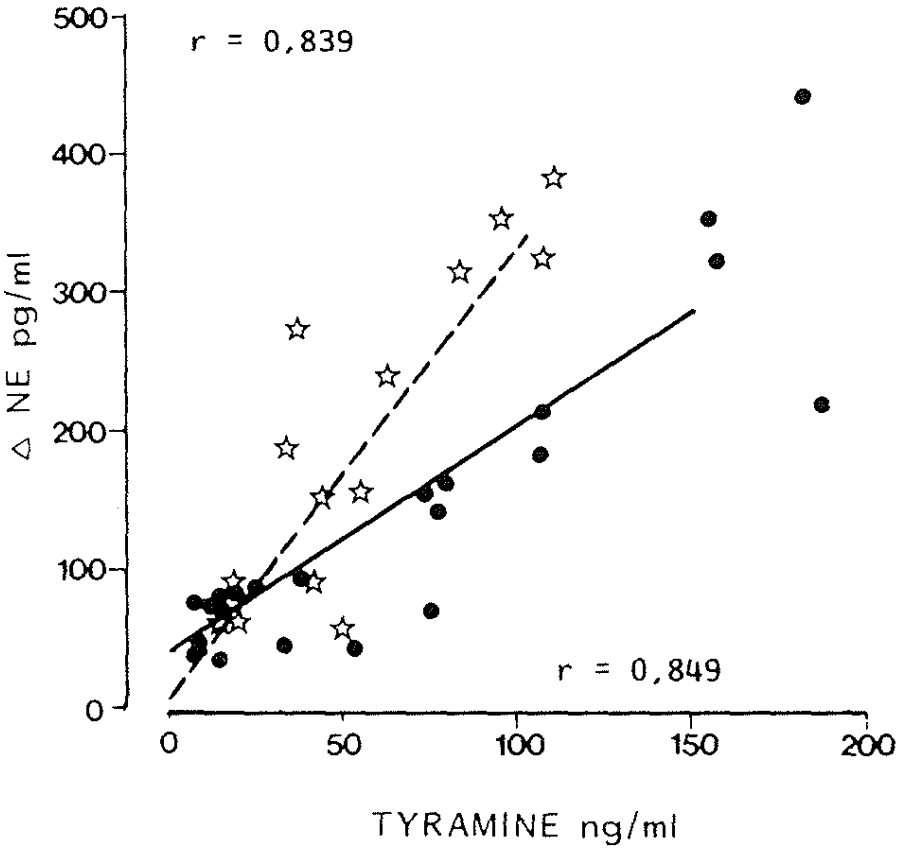

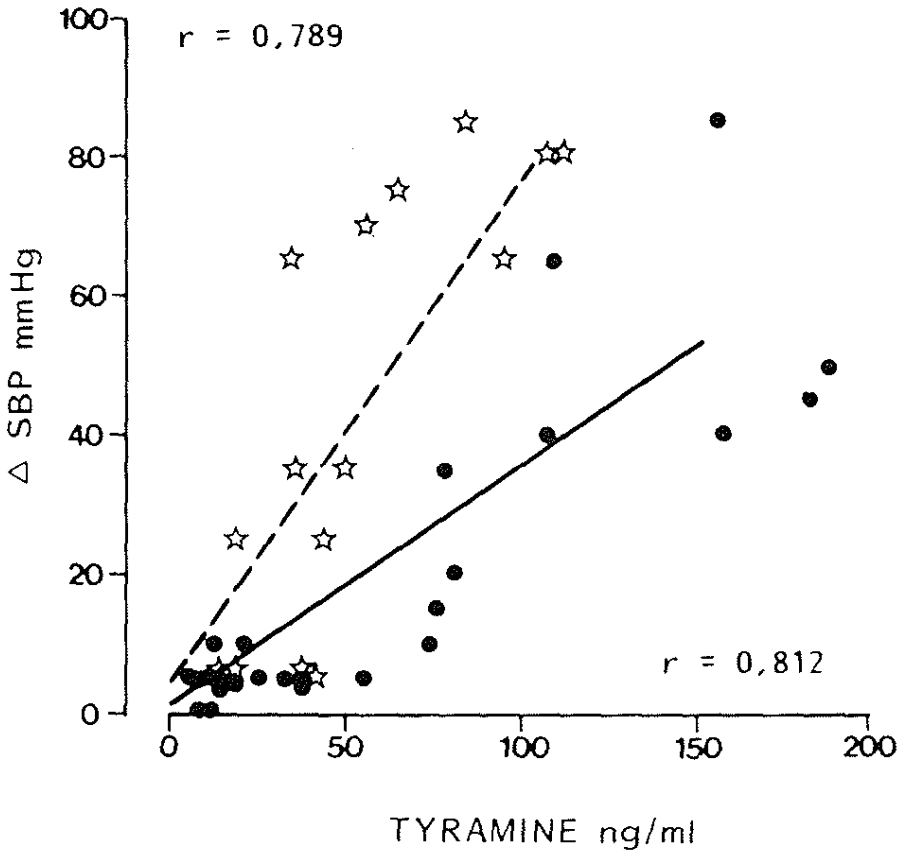

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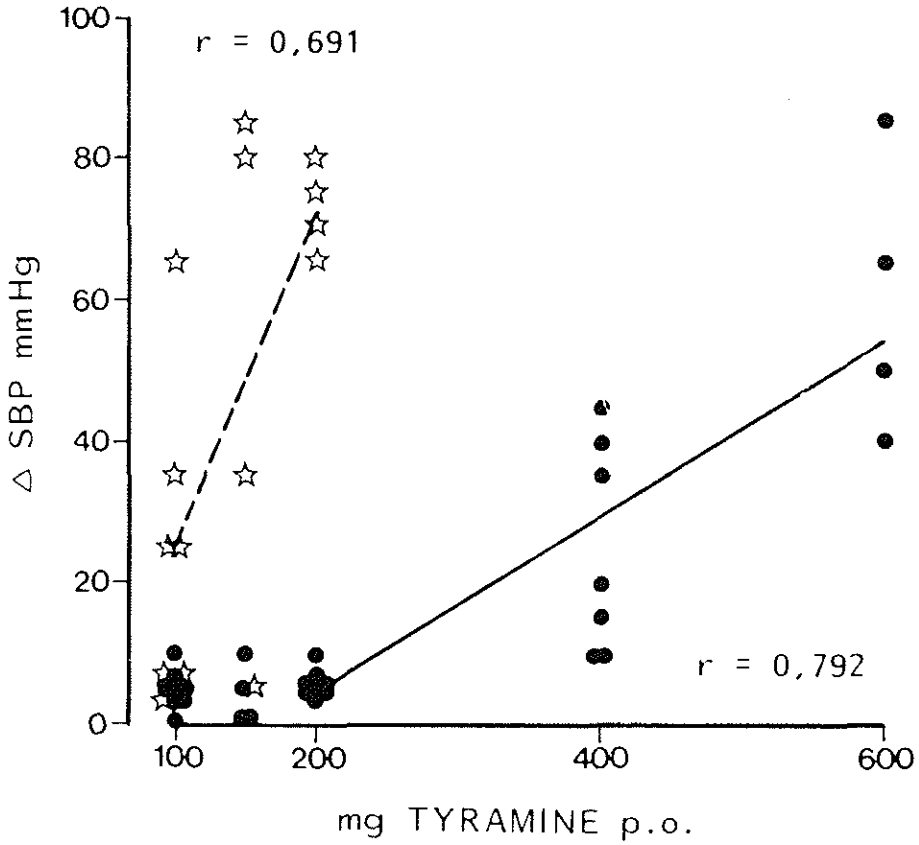

Tyramine Potentiation: Role of MAO-A (and MAO-B)

Potentiation of tyramine response (increase in potency of tyramine to raise blood pressure by 30 mmHg)

- Moclobemide (300 mg b.i.d.): 3-4 fold

- Moclobemide (300 mg b.i.d.) plus lazabemide (100 mg b.i.d., reversible selective MAO-B inhibitor): 13.5 fold

- Tranylcypromine (irreversible non-selective MAO A/B inhibitor): 20-40 fold

Dingemanse et al. 1996 Int J Clin Pharmacol Ther 34:172-177

Adverse Event: due to IMP or NIMP?

- Causality of AEs split into IMP and NIMP
- AE section of CRF needs adaptation to causality related to two compounds

Main Risks of Tyramine

- Excessive increase in blood pressure
- Headache, dizziness, restlessness, palpitations, forceful heartbeat, dry mouth, gastric discomfort, nausea, vomiting, pallor, regional vasoconstriction, shortness of breath, tightness of chest or neck, precordial distress, chest throbbing
- Arrhythmias (sinus arrhythmias such as bradycardia, AV block and tachycardia, atrial extrasystoles; junctional rhythm, Wenckebach periods, ventricular arrhythmias such as premature beats, couplets, bigeminy, ventricular extrasystoles)
- AEs related to increase in noradrenaline release, therefore transient and sensitive to alpha-adrenoceptor antagonists (phentolamine)

Emergency Treatment of Excessive Tyramine Response

Rescue Medication

- Supplies (intravenous fluids and/or medications).
- Phentolamine (alpha-adrenoceptor antagonist) to treat hypertensive crises or pronounced reactive bradycardia due to baroreceptor activation followed by vagal hyperactivity and may be used to instantaneously antagonize the tyramine effect on BP or arrhythmias
- Phentolamine (Regitin®, Novartis Pharma, Basle, Switzerland) administered intravenously with cautious titration as rescue medication, if

• the tyramine pressor response exceeds 60 mmHg, or

• if the SBP exceeds 180 mmHg, or

• if the pulse falls below 40 beats per minute (bpm), or

• if arrhythmias are considered to be of threatening extent, or

• other criteria are possible at the discretion of the Investigator

- Phentolamine to be slowly injected should not exceed 5 mg, unless no reversal of the tyramine-induced effects is seen after about 5 minutes. In this case cautiously further amounts (5 mg or less) of phentolamine can be administered by slow injection.
- Alternatively, if phentolamine cannot be given, intravenous nicardipine or urapidil chlorhydrate may be used as rescue medication.

Serious Adverse Events (SAEs)

- Possibly related to interaction between IMP and NIMP, and unexpected:

  SUSAR

- Possibly attributed to IMP or NIPM:

  SUSAR

- Definitely due to NIMP and likely to affect safety of trial subjects:

  Expedited reporting

Summary

- Challenge study: a real challenge not just for the healthy volunteer, but also for sponsor and investigator
- Carefully balance risk and benefit to avoid damage

Thank You for Your Interest