HAL open science

Characterization of platelet-activating factor receptors in circular smooth muscle of guinea-pig ileum by (3H)PAF binding

Rafael Garcia Villar, Olivier Jeanneton, Alain Botella, Jean-Pierre Martinolle, Michel Delvaux, Lionel Bueno

To cite this version:

Rafael Garcia Villar, Olivier Jeanneton, Alain Botella, Jean-Pierre Martinolle, Michel Delvaux, et al.. Characterization of platelet-activating factor receptors in circular smooth muscle of guinea-pig ileum by (3H)PAF binding. Digestive Disease Week/95th AGA meeting, May 1994, New-Orleans, Louisiana, United States. 1994, Gastroenterology 106 (4). hal-01601035

HAL Id: hal-01601035

https://hal.science/hal-01601035v1

Submitted on 4 Jun 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution - ShareAlike 4.0 International License

ABSTRACT FORM FOR: **American Gastroenterological Association**
May 15-18, 1994 **American Association for the Study of Liver Diseases**
New Orleans, Louisiana

Type name, address, and telephone numbers of author who should receive correspondence in Box F and complete all other parts of the form. ABSTRACTS MUST BE REPRODUCIBLE

CHARACTERIZATION OF PLATELET-ACTIVATING FACTOR (PAF) RECEPTORS IN CIRCULAR SMOOTH MUSCLE OF GUINEA PIG ILEUM BY [3H]PAF BINDING.

R. Garcia-Villar, O. Jeanneton, A. Botella, J.P. Martinolle, M. Delvaux & L. Buéno. INRA, Department of Pharmacology, BP.3, 31931 Toulouse, France.

Platelet-activating factor (PAF), a key component intestinal inflammation, exerts contractile effects on freshly dispersed smooth muscle cells from guinea pig ileum circular layer (JPET 1993;267:31-37). Although these data suggest a direct effect of PAF, evidence for the presence of specific receptors in this cell type is lacking. The present study was therefore aimed at identifying and characterizing PAF receptors in membrane fractions prepared from these smooth muscle cells.

Methods. Binding saturation studies were performed by incubating [3H]PAF (0.01 to 10 nM) with 60-100 µg of membrane proteins for 15 min at 20°C in 50 mM Tris-HCl, 10 mM $MgCl_2$ buffer (pH 7.0), containing aprotinin (10^3 IU/ml) and PMSF (200 µM). Binding competition studies were performed with selected agents (10^{-13} to 10^{-3} M) i.e. the PAF receptor antagonists SR 27417 (Sanofi, F), L659.989 (MSD, USA), BN 52021 (Beaufour, F), and the agonist *DL*-PAF-C16, but also acetylcholine (Ach), prostaglandin (PG) E2 and sulfated cholecystokinin octapeptide (CCK8S).

Results. [3H]PAF binding to our membrane preparations was a saturable process of high affinity (Bmax = 1062 ± 456 fmol/mg of protein ; K_D = 2.3 ± 0.8 nM ; mean±SD, n=11). Non-specific binding, determined in presence of 10 µM unlabeled PAF, increased linearly with [3H]PAF concentration and, at a radioligand concentration close to its K_D, accounted for 41 to 67% of total binding determined in the absence of unlabeled PAF. The analysis of saturation binding isotherms by both Scatchard (Hill coefficient not different from unity) and non-linear regression (LIGAND) procedures, suggested the existence of only one class of binding sites. However, binding of [3H]PAF (4 nM) at a steady state appeared to be inhibited by the selected antagonists (n=3) BN 52021 (IC_{50} = 0.33 ± 0.17 nM), SR 27417 (IC_{50} = 0.71 ± 0.15 nM), L659.989 (IC_{50} = 0.66 ± 0.39 nM) as well as by unlabeled PAF (IC_{50} = 3.71 ± 0.93 nM ; n=6) but not by Ach, PGE2 and CCK8S (IC_{50} > 105 nM ; n=3); the shallow-shaped inhibition curves (slope factor < 1; $p<0.05$) suggested either an interaction of [3H]PAF with more than one specific PAF receptor-subtype, or the presence of different affinity states of the same PAF receptor in our membrane preparation.

Conclusion. These data demonstrate the presence of specific PAF receptors in ileum smooth muscle cells and support a direct myogenic action of PAF in the intestinal motility changes observed during gut inflammation.

A. ABSTRACT DESCRIPTOR
A numbered list of abstract descriptors appears on the reverse of this form. Please enter below the number of the descriptor you have circled that best fits the subject of your abstract. Select **one** descriptor only.

Descriptor number 65

B. POSTER ONLY
Check here ☐ if you want your abstract presented only as a poster.

C. PRIZES AND AWARDS
Check the appropriate box if you are applying for an AGA Foundation Student Abstract Prize, AGA Foundation International Travel Award, or AGA World Congress of Gastroenterology Travel Award.

☐ Student Abstract Prize
☐ International Travel Award
☐ World Congress Travel Award

D. CONFLICT OF INTEREST DISCLOSURE
Check the appropriate box:

☒ No potential conflict of interest exists.
☐ A potential conflict of interest exists. A letter of explanation is enclosed.

E. DDW INFORMATION
Check here ☒ if you are not a member of AGA or AASLD and would like to receive meeting registration information.

F. Name GARCIA-VILLAR, RAFAEL
Address INRA - Department of Pharmacology-Toxicology
180 chemin de Tournefeuille - BP 3
31931 TOULOUSE CEDEX - FRANCE
Telephone (Office) 33 61 28 51 51 (Fax) 33 61 28 53 10

SUBMIT ABSTRACT PLUS TWO PHOTOCOPIES TO:
AGA
c/o PRISM Productions
5040 Pine Creek Drive
Westerville, Ohio 43081-4821

I (senior author) affirm that this abstract meets all of the requirements stated in this booklet, that the authors named are familiar with the presented data, and that box D has been completed.

Signed Garcia-Villar Rafael

● ORNITHINE DECARBOXYLASE ACTIVITY IN RAT INTESTINAL MUCOSA WAS STIMULATED BY SIGNALS OF CENTRAL NERVOUS SYSTEM.

K. Fujimoto, J. Tanaka, H. Morita, H. Sakata, R. Iwakiri. Div. of Gastroenterol., Dept. of Intern. Med., Saga Med. School, Saga 849, JAPAN.

Feeding plays an important role in regulating ornithine decarboxylase (ODC) activity in small intestine, because ODC activity is increased by feeding mediated through luminal nutrients and humoral factors. The aim of this study is to determine whether ODC activity in rat small intestine is controlled by signals from the central nervous system other than luminal nutrients.

Method. Subdiaphragmatic vagotomy was accomplished under halothane anesthesia one week before experiments. In sham control rats, the vagal fibers were isolated but not cut. ODC activity in intestinal mucosa of ad libitum fed rats or 24 h fasted rats was measured at 4 time points (05:00, 11:00, 17:00, 23:00 h; light period: 08:00 to 20:00 h). In additional rats of 24 h fasted rats, ODC activity in small intestinal mucosa and liver was measured after intrathird ventricular infusion of 24 μmol 2-deoxy-glucose at 11:00 h.

Results. *Table. ODC activity (CO_2 pmol/mg protein/h) in duodenal and jejunal mucosa of 24 h fasted rats.*

	11:00	17:00	23:00	05:00
DUODENUM				
Sham	1.0±1.1	26.2±1.1*	4.3±1.9	4.4±1.9
Vagotomy	0.9±0.9	2.2±1.2#	3.2±2.0	3.2±1.9
JEJUNUM				
Sham	1.3±0.7	2.3±0.9	24.4±4.2*	26.9±3.2*
Vagotomy	1.5±0.7	1.8±1.0	23.0±2.8*	25.1±3.0*

*=P<0.01, compared to the value at 11:00 h. #=P<0.01, compared to the value of sham.

ODC activity in the jejunum increased in the dark period when rats eat. In contrast, ODC activity in the duodenum began to increase at 17:00 h when rats do not normally eat. The increase of ODC activity in the duodenum at 17:00 h remained in 24 h fasted rats (Table). The increase in ODC activity in the duodenum at 17:00 h, but not at other time points, was abolished by subdiaphragmatic vagotomy both in ad libitum fed and 24 h fasted groups, whereas vagotomy had no effect on the feeding pattern of the rat. Subdiaphragmatic vagotomy had no effect on ODC activity in the jejunum of the both groups. Infusion of 2-deoxy-glucose into the cerebroventricle induced glycoprivation of the central nervous system (CNS) and activated neural activity of lateral hypothalamus, which increased ODC activity in the small intestine and liver. Vagotomy attenuated the increase of ODC activity after infusion of 2-deoxy-glucose, but did not abolish. Conclusions. The CNS, in addition to liminal nutrient factors, plays an important role, at least in part, via the efferent vagal nerve in the increase in intestinal mucosal ODC activity.

● **ATP AND 5-HT MEDIATE FAST EXCITATORY POSTSYNAPTIC POTENTIALS (fEPSPS) IN MYENTERIC NEURONS OF GUINEA PIG ILEUM.** J.J. Galligan, Dept. of Pharmacol., Michigan State University, E. Lansing, MI 48824, USA.

Acetylcholine (ACh), acting at nicotinic receptors, is the principle fast excitatory neurotransmitter in the gut but ATP and 5-HT can mimic fEPSPs. Receptors for ATP and 5-HT are present on myenteric neurons but it is unclear if ATP or 5-HT contribute to fEPSPs. The purpose of these studies was to identify noncholinergic fEPSPs in myenteric neurons. Conventional electrophysiological methods were used to record from neurons in the myenteric plexus. Drugs were applied by superfusion, iontophoresis or pressure ejection from a pipette positioned near impaled neurons; fEPSPs were evoked by single focal stimuli (0.5 ms, 0.2 Hz). Control and drug-treated responses were a computer average of 8-12 fEPSPs. Membrane potential was adjusted to -90 mV to limit action potentials. Data were obtained from 31 neurons exhibiting fEPSPs (mean = 21 ± 1 mV). In 7 cells, fEPSPs were reduced more than 80% by hexamethonium (C6, 100 μM); in 24 cells, fEPSPs were reduced by only 34 ± 4%. C6-resistant responses were completely blocked by tetrodotoxin (0.3 μM). In 13 cells, C6-resistant fEPSPs were reduced by the P_2 receptor antagonist suramin. Suramin at 3, 10, 30, 100 and 300 μM reduced C6-resistant fEPSPs by 30 ± 7%, 49 ± 5%, 66 ± 8%, 68 ± 5% and 77 ± 6%, respectively. The 5-HT_3 receptor antagonist, ondansetron (1 μM) reduced C6-resistant fEPSPs more than 70% in 11 neurons. C6 blocked ACh depolarizations but not those induced by ATP or 5-HT. Ondansetron (1 μM) blocked 5-HT depolarizations but not those caused by ACh or ATP. Suramin (100 μM) did not affect ACh or 5-HT responses. These data indicate that ACh, 5-HT, and ATP are mediators of fEPSPs in myenteric neurons of guinea pig ileum. (Supported by DK40210)

● CHARACTERIZATION OF PLATELET-ACTIVATING FACTOR (PAF) RECEPTORS IN CIRCULAR SMOOTH MUSCLE OF GUINEA PIG ILEUM BY [3H]PAF BINDING.

R. Garcia-Villar, O. Jeanneton, A. Botella, J.P. Martinolle, M. Delvaux & L. Buéno. INRA, Department of Pharmacology, BP.3, 31931 Toulouse, France.

Platelet-activating factor (PAF), a key component intestinal inflammation, exerts contractile effects on freshly dispersed smooth muscle cells from guinea pig ileum circular layer (JPET 1993;267:31-37). Although these data suggest a direct effect of PAF, evidence for the presence of specific receptors in this cell type is lacking. The present study was therefore aimed at identifying and characterizing PAF receptors in membrane fractions prepared from these smooth muscle cells.

Methods. Binding saturation studies were performed by incubating [3H]PAF (0.01 to 10 nM) with 60-100 μg of membrane proteins for 15 min at 20°C in 50 mM Tris-HCl, 10 mM $MgCl_2$ buffer (pH 7.0), containing aprotinin (103 IU/ml) and PMSF (200 μM). Binding competition studies were performed with selected agents (10-13 to 10-3 M) i.e. the PAF receptor antagonists SR 27417 (Sanofi, F), L659.989 (MSD, USA), BN 52021 (Beaufour, F), and the agonist *DL*-PAF-C16, but also acetylcholine (Ach), prostaglandin (PG) E2 and sulfated cholecystokinin octapeptide (CCK8S).

Results. [3H]PAF binding to our membrane preparations was a saturable process of high affinity (Bmax = 1062 ± 456 fmol/mg of protein ; K_D = 2.3 ± 0.8 nM ; mean±SD, n=11). Non-specific binding, determined in presence of 10 μM unlabeled PAF, increased linearly with [3H]PAF concentration and, at a radioligand concentration close to its K_D, accounted for 41 to 67% of total binding determined in the absence of unlabeled PAF. The analysis of saturation binding isotherms by both Scatchard (Hill coefficient not different from unity) and non-linear regression (LIGAND) procedures, suggested the existence of only one class of binding sites. However, binding of [3H]PAF (4 nM) at a steady state appeared to be inhibited by the selected antagonists (n=3) BN 52021 (IC50 = 0.33 ± 0.17 nM), SR 27417 (IC50 = 0.71 ± 0.15 nM), L659.989 (IC50 = 0.66 ± 0.39 nM) as well as by unlabeled PAF (IC50 = 3.71 ± 0.93 nM ; n=6) but not by Ach, PGE2 and CCK8S (IC50 > 105 nM ; n=3); the shallow-shaped inhibition curves (slope factor < 1; p<0.05) suggested either an interaction of [3H]PAF with more than one specific PAF receptor-subtype, or the presence of different affinity states of the same PAF receptor in our membrane preparation.

Conclusion. These data demonstrate the presence of specific PAF receptors in ileum smooth muscle cells and support a direct myogenic action of PAF in the intestinal motility changes observed during gut inflammation.

● IDENTIFICATION OF SELECTIVE AGONISTS AND ANTAGONISTS FOR HUMAN BOMBESIN RECEPTORS USING STABLY TRANSFECTED CELL LINES.

F. Gibril, J.E. Mrozinski, T. Griffin, D.H. Coy, R.V. Benya, J.F. Battey, R.T. Jensen. NIH, Bethesda, MD and Tulane University, New Orleans, LA

The mammalian BN-related peptides gastrin-releasing peptide (GRP) and neuromedin B (NMB) as well as their receptors, are widely distributed in the CNS and GI tract. It has been proposed these peptides mediate numerous functions such as control of temperature, growth, release of many GI hormones, motility, pancreatic secretion and lung development. However, their physiological importance remains unclear in humans because of lack of specific information on the pharmacology of the human receptors. In the present study we have made stable cell lines containing hGRP receptors (hGRP-R) and NMB receptors (hNMB-R) by transfecting Balb 3T3 cells with full length human receptor clones. Interaction with hGRP-R was assessed using ^{125}I-[Tyr^4]BN and with hNMB-R with ^{125}I-[Tyr^0]NMB. Receptor activation was measured by determining changes in [^{3}H]IP. Ten different naturally occurring BN-related agonists and 26 different antagonists representing the 6 known classes of BN-receptor antagonists were assessed. For hGRP-R, BN, alytesin, ranatensin, PG-L and litorin, all had equal high affinity (1nM) >GRP>>NMB, NMB-32. With hNMB-R, NMB, NMB-32, litorin, all had equal high affinity>BN, alytesin>>GRP. Changes in [^{3}H]IP showed a similar result. In terms of selectivity, the agonists fell into 3 groups; GRP, NMC had>150-fold higher affinity for hGRP-R, NMB had>60-fold higher for hNMB-R, and ranatensin, alytesin, litorin had relative high affinity for each. Each antagonist had no agonist activity at concentrations up to 3μM. For hGRP-R the highest affinity antagonists were [D-F$_5$-Phe^6,D-Ala^{24}]BN(6-13)methyl ester, [D-Phe^6]BN(6-13)ethyl amides, *N*-Ac-GRP(20-26)ethyl ester, [D-Phe^{19},D-Ala^{24},ψ26-27,D-Pro^{26}, Pro^{27})GRP(19-27),[D-Phe^6,ψ13-14,Cpa^{14})BN(6-14)K$_i$(0.9-3nM)>>[D-Arg^1,D-Trp^{7,9},Leu^{11}]SP>[Tyr^4,D-Phe^{12}]BN. For hNMB-R, no antagonists had<1μM affinity. Similar results were obtained on studies of the ability of these analogues to inhibit [^{3}H]IP stimulated by BN or NMB. Each of the high affinity hGRP analogues had>500-fold selectivity for hGRP-R. These results show for the first time the pharmacology of human BN receptors. In general, they closely resemble results with the guinea pig and mouse but differ from the rat. These results demonstrate GRP is much more selective for hGRP-R and NMB for hNMB-R than any of the nonmammalian peptide agonists. Furthermore, a number of potent highly selective hGRP-R, but not hNMB-R antagonists exist will should be useful for exploring the function of this receptor in vivo.

April 1994 Volume 106 • Number 4

Supplement to

Gastroenterology

Digestive Disease Week and the 95th Annual Meeting of the American Gastroenterological Association

May 15-18, 1994, New Orleans, Louisiana

Program of the Annual Meeting of the American Gastroenterological Association, the American Association for the Study of Liver Diseases, the Gastroenterology Research Group, the Society for Surgery of the Alimentary Tract, and the American Society for Gastrointestinal Endoscopy

Abstracts of Papers Submitted to the American Gastroenterological Association

Abstracts of Papers Submitted to the American Association for the Study of Liver Diseases

Student Research Abstracts