中国药理学报 1989年3月；10（2）：111-114

木犀草素-组胺和受体反应的药效动力学

沈企华 （第二军医大学训练部药理学教研室，上海 200433）

Acta Pharmacologica Sinica 1989 Mar; 10 (2): 111-114

Pharmacodynamics of luteolin-histamine and recepors response

SHEN Qi-Hua (*Second Military Medical College, Shanghai 200433*)

Abstract The isolated guinea pig ileums were used to investigate the pharmacodynamics of the relationship between luteolin and histamine dose response. The pharmacodynamics parameters were estimated by various methods. The values of the pA_2, the slope and the pD_2' were 5.6, 0.6 and 4.4, respectively. The dissociation constants and the maximal response were estimated by the Lineweaver-Burk, Hanes-Woolf, Scatchard or Woolf methods. The estimated values of the parameters were: $E_{A\max} \neq E_{AB\max}$, $K_A \neq \alpha K_A$, $K_B \neq \alpha K_B$. The linear intersecting points of the plotting by the Lineweaver-Burk method were above X axis (2nd or 1st quadrant), by the Hanes-Woolf method were below X axis (3rd quadrant), by the Scatchard method were below X axis (4th quadrant) and by the Woolf method were to the left of Y axis (2nd quadrant). The antagonistic action of luteolin is assumed to be noncompetitive and competitive in combination. The estimated Hill coefficient approached to 1 which is accorded with the M-M formula.

Although any one of the four linear transformation method of the M-M equation could be used to estimate the dissociation constants and the maximal responses, there exists difference between them. The values of the parameters obtained by the Lineweaver-Burk method were excessively large (eg, $E_{AB_2\max} = 360.3$, $K_{AB_2} = 28.4$), even negative (eg, $\alpha K_{B_1} = -7.9$, $\alpha K_{B_2} = -14.6$), and so it is inferior to the other three methods, as reported in the literature. The values of the parameters obtained by the Hanes-Woolf and the Woolf method were rather close and were superior to the Scatchard method, which is in accord with the literature reports.

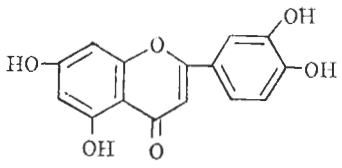
Key words luteolin; histamine; drug receptors; pharmacodynamics; ileum; dissociation constant; drug dose-response relationship

提要 用离体豚鼠回肠进行了木犀草素-组胺和受体反应的药效动力学研究。实验求得量效关系曲线，用不同方法转变成直线，各直线交点所示及求得的动力学参数：$E_{A\max} \neq E_{AB\max}$，$K_A \neq K_B$，$K_B \neq \alpha K_B$，表明木犀草素拮抗组胺是属于非竞争性与竞争性的混合型拮抗作用，求得Hill系数近于1，说明它符合M-M方程式型式的受体动力学原理。

关键词 木犀草素；组胺；药物受体；药效学；回肠；解离常数；药物剂量-效应关系

木犀草素（luteolin）属黄酮类化合物，它对组胺与过敏性慢反应物质（SRA-A）[1]具有拮抗作用。为了弄清其对组胺拮抗作用的性质，用离体回肠进一步实验，将实验数据根据药物与受体反应的药效动力学原理[2]，用不同方法（Lineweaver-Burk, Hanes-Woolf, Scatchard and Woolf）[3-5]作图及计算药效动力学参数，来分析木犀草素对组胺拮抗作用的性质。

1988年1月30日收稿 1988年10月14日接受



Luteolin

Materials and methods

木犀草素由本校药学系提供，以适量吐温80混匀助溶，吐温80含量达到2%为止，配成所需浓度，调节pH为7.0-7.5。磷酸组胺系中国科学院生物化学研究所产品。

实验方法 采用离体豚鼠回肠实验装置。浴管容量为10 ml，通95%O_2+5%CO_2，浴温保持32±0.5℃，取回肠一段，一端固定在钢丝钩上，另端通过杠杆与张力换能器连接，静止张力为0.5 g，用自动平衡记录仪记录加入组胺-木犀草素的回肠收缩曲线，组胺用累积剂量给药法，在浴管中的浓度为0.3，1，10和30 μmol/L，使肠肌收缩达到最大效应(100%)为止，得累积剂量效应%。作为对照，然后用木犀草素3 μmol/L与肠肌作用2 min，接着用以上不同剂量的组胺，得木犀草素-组胺累积剂量效应%。再依次分别测定木犀草素10和30 μmol/L的组胺累积剂量效应%。

Results

组胺(A)及木犀草素(B)-组胺累积剂量对豚鼠回肠收缩效应%(见Tab 1)。

从剂量-效应曲线及用Arunlakshana-Schild[6]作图法估计出$pA_2=5.6$，其斜率(b)为−0.6，说明其不属于竞争性拮抗作用。用Ariëns-van Rossum[7]公式估计出pD'_2为4.4；以Michaelis-Menten (M-M)方程式型式的双曲线函数公式[1]和公式[2]：

$$E_A = E_{Amax}[A]/K_A+[A] \qquad [1]$$

$$E_{AB} = \frac{E_{Amax}[A]}{K_A(1+[B]/K_B)+[A](1+[B]/\alpha K_B)} \qquad [2]$$

Tab 1. Contraction (%) of guinea pig ileum (number in parentheses) after histamine and luteolin. $\bar{x}\pm SD$. $^*P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$

Histamine (μmol/L)	Luteolin (μmol/L) 0 (23)	3 (5)	10 (12)	30 (6)
0.3	13±10	5.7±2.7*	4±3***	2.7±2.4**
1.0	34±13	21±7**	16±9***	6±4***
3.0	68±11	49±7***	40±15***	23±10***
10.0	91±6	75±9***	59±14***	48±16***
30.0	100±0	83±12***	69±16***	54±14***

式中E_{Amax}为全部受体(R)被占领时所产生的最大效应；K_A为复合物(RA)的解离常数；[]表示药物的浓度；K_B为拮抗剂(B)的复合物(RB)的解离常数；αK_B为RBA复合物的解离常数；激动剂(A)从RBA上解离常数为αK_A。αK_A实为K_A增大$(1+[B]/K_B)$倍，即$K_A(1+[B]/K_B)=\alpha K_A$

将以上两公式各转变成4种直线方程式，用最小二乘法原理回归运算，不管那种直线方程式所求得的动力学参数均各不相同：$K_A \neq K_B$，$K_A \neq \alpha K_A$，$K_B \neq \alpha K_B$，$E_{Amax} \neq E_{ABmax}$(见Tab 2)。

用Lineweaver-Burk式作图所示：组胺累积剂量对豚鼠回肠收缩效应直线A与加木犀草素后组胺效应直线的斜率随(B)增加而增加，A直线与AB_1，AB_2直线交点在第1象限，而AB_3直线与A直线交点在第2象限。总之，其交点都在横轴以上(见Fig 1A)。用Hanes-Woolf (Scott)式即量效比式作图所示：直线斜率也都增大了，直线的交点在横轴以下(第3象限内)(见Fig 1B)。用Scatchard式作图所示：直线交点在横轴以下(第4象限内)(见Fig 1C)。用Woolf (Eadie-Hofstee)式作图所示：其直线交点在纵轴以左(第2象限内)(见Fig 1D)。从以上4种直线方程式求得的药效动力学参数及作图所示都符合于非竞争性与竞争性的混合型拮抗作用原理[3-5]，如果仅由于受体分子空间构象的改变来调节受体的活力，以Tab 1,2的

Tab 2. The estimated values of pharmacodynamics parameters from data in Tab 1 and the formula [1],[2] by the Lineweaver-Burk (L-B), Hanes-Woolf (H-W), Scatchard (S) and Woolf (W) method. K = dissociation constant, E_{max} = maximal response (%), A = histamine, B 1, 2, 3 = luteolin 3, 10, 30 μmol/L.

	L-B	H-W	S	W
Parameters from formula [1]				
E_{Amax}(%)	112.7	106.8	111.1	110.6
K_A(μmol/L)	2.3	1.9	2.2	2.2
E_{AB1max}(%)	182.4	93.3	108.4	99.6
K_{AB1}(μmol/L)	9.2	3.4	4.6	3.9
E_{AB2max}(%)	360.3	79.4	95.2	79.1
K_{AB2}(μmol/L)	28.4	4.3	5.9	4.2
E_{AB3max}(%)	53.2	69.1	84.6	69.7
K_{AB3}(μmol/L)	5.7	7.2	9.7	7.2
Parameters from formula [2] (μmol/L)				
K_{B1}	2.1	2.9	2.6	3.0
αK_{A1}	5.7	3.9	4.7	4.4
αK_{B1}	−7.9	22.1	90.7	27.3
K_{B2}	3.5	4.9	4.7	5.9
αK_{A2}	4.3	5.8	6.9	5.9
αK_{B2}	−14.6	29.1	57.2	25.2
K_{B3}	7.0	6.2	6.2	7.1
αK_{A3}	12.2	11.1	12.8	11.5
αK_{B3}	29.4	54.9	94.3	51.1

数据，用 Hill 方程式[3]，求出 n_H，结果 n_H 值近于 1(见 Tab 3)，说明它是符合 M-M 方程式型式的受体动力学原理。

$$\log E/E_{max}-E = n\ \log[A] - \log K_A \qquad [3]$$

Tab 3. Hill number (n_H).

Method of E_{max}	E_A	E_{AB_1}	E_{AB_2}	E_{AB_3}
Hanes-Woolf	0.90	1.07	1.06	1.05
Scatchard	0.93	0.91	0.91	0.94
Woolf	1.18	0.99	1.07	1.05
$\bar{x}$	1.00	0.99	1.01	1.01
±SD	±0.15	±0.08	±0.09	±0.06

Discussion

本人曾用 Lockett MF[8]等实验设计求出组胺及木犀草素的 pA_2 为 4.6±0.12，不能说明是属于单纯性竞争性拮抗作用。本实验证明是属于非竞争性与竞争性的混合型拮抗作用。有些学者[9,10]认为非竞争性拮抗的条件 $K_A = \alpha K_A$，$K_B = \alpha K_B$ 是很少可能存在的，只改变 E_{Amax} 而不改变 K_A 的拮抗剂，只可能是小分子的物质。故 $K_B = \alpha K_B$ 的拮抗可看作是混合型拮抗中一个非常特殊的例子，因此有人把非竞争性拮抗作用与混合型拮抗作用合并成一类。事实上，从药物与受体结合所产生的效应推导出非竞争性拮抗作用公式[2]，如果 $K_B = \alpha K_B$，则

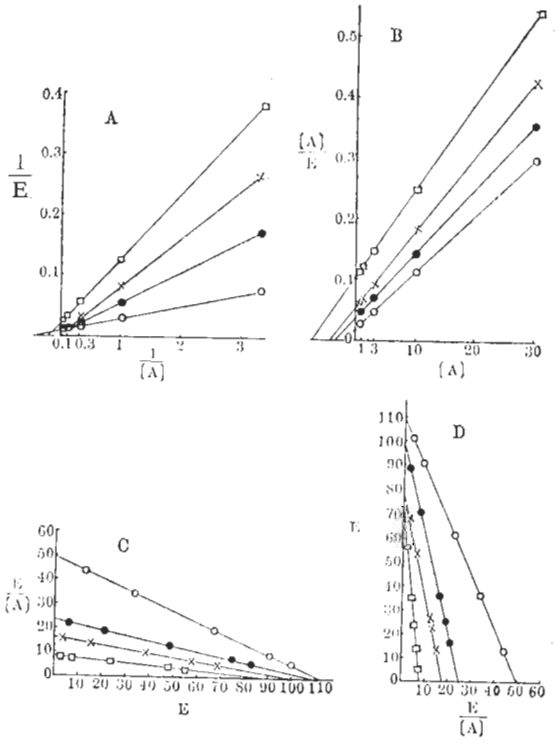


Fig 1. The effective data (Tab 1) of luteolin-histamine on receptor response, plotting linear transformation of the formula [1] and [2] by the A) Lineweaver-Burk ($1/E$ *vs* $1/[A]$), B) Hanes-Woolf ($[A]/E$ *vs* $[A]$), C) Scatchard ($E/[A]$ *vs* E) and D) Woolf (E *vs* $E/[A]$) methods. The linear intersecting points: A) in second or first quadrant, B) in third quadrant, C) in fourth quadrant, D) in second quadrant. (○) A, (●) AB_1 (×) AB_2, (□) AB_3.

$$E_{AB} = \frac{E_{Amax}[A]}{(K_A + [A])(1 + [B]/K_B)} \qquad [4]$$

实际上，公式[2]就是混合型拮抗作用的公式。

从 4 种直线方程式运算所得的药效动力学参数，以 Lineweaver-Burk 式偏差最大，与文献报道[11,12]一致，如 E_{AB_2max}，K_{AB_2} 等数值过大，并且出现 αK_{B_1}，αK_{B_2} 为负值，以 Hanes-Woolf 和 Woolf 二式所求得的动力学参数基本上比较接近，Scatchard 式次于 Hanes-Woolf 和 Woolf 式，如出现 αK_{B_1}，αK_{B_3} 数值较大，Scatchard 式次于量效比式，正如金正均指出[13]不涉及多种受体时，即 $n_H = 1$ 时，量效比式较 Scatchard 式更为适合。

致谢 本校药学系韩公羽教授提供木犀草素。

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