Blunting of diuretic-induced increases in urinary magnesium and potassium outputs by beta-adrenergic blockade in healthy subjects

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Zusammenfassung

Der Zweck dieser Studie lag darin festzustellen, ob eine adrenerge β-Blockade einen Einfluß auf die Diuretika-bedingte Hypermagnesiurie und Hyperkaliurie hat.

Sieben Tage lang wurde an neun gesunden Versuchspersonen Plazebo, das Diuretikum Clopamid 5 mg (CLOP), der adrenerge β-Blocker Pindolol 10 mg (PIND) und eine Kombination von CLOP und PIND (CLOP + PIND) im randomisierten Doppelblind-Versuch verabreicht. Der Stoffwechsel der Personen wurde unter streng kontrollierten Bedingungen untersucht.

CLOP und CLOP + PIND erhöhen die Flüssigkeits- und Natrium-Ausscheidung über den 24-Stunden-Urin signifikant im Vergleich zur Plazebo-Gruppe. CLOP erhöhte signifikant die Mehrausscheidung von Mg^{2+} ($\%\Delta = 27$, $p<0{,}01$) und K^+ ($\%\Delta = 25$, $p<0{,}05$) im 24-Stunden-Urin im Vergleich zur Plazebo-Gruppe während CLOP + PIND keine der Ausscheidungen signifikant beeinflußte ($\%\Delta$ für $Mg^{2+} = -1$; $\%\Delta$ für $K^+ = -10$). PIND beeinflußte keine der renalen Ausscheidungen signifikant.

CLOP könnte die renale Produktion von Renin erhöhen, erhöht aber gleichzeitig die Plasmaaldosteron-Konzentration. PIND könnte diese Erhöhung abgeschwächt haben. Daher könnte der Diuretika-bedingte Anstieg der Mehrausscheidung von Mg^{2+} über die Niere zumindest teilweise auf einen Hyperaldosteronismus zurückzuführen sein. Die möglichen Vorteile dieser Ergebnisse für die Behandlung von Hypertonie-Patienten müssen in Langzeit-Studien untersucht werden.

Summary

The objective of this study was to assess whether beta-adrenergic blockade influences diuretic-induced hypermagnesiuresis and hyperkaliuresis.

Single doses of placebo, of the diuretic clopamide 5 mg (CLOP), of the beta-adrenergic blocker pindolol 10 mg (PIND) and of a combination of CLOP and PIND (CLOP + PIND) were administered 7 days apart, double-blind and in random order, to nine healthy volunteers. Subjects were studied in a metabolic ward under strictly controlled conditions.

CLOP and CLOP + PIND significantly increased the mean 24-h urinary outputs of fluid and Na^+ with respect to placebo. CLOP significantly increased the mean 24-h urinary outputs of Mg^{2+} ($\%\Delta = 27$, $p<0{,}01$) and K^+ ($\%\Delta = 25$, $p<0{,}05$) with respect to placebo, whereas CLOP + PIND did not affect either output significantly ($\%\Delta$ for $Mg^{2+} = -1$; $\%\Delta$ for $K^+ = -10$). PIND did not affect any of the studied urinary outputs significantly.

CLOP would have augmented the renal production of renin and subsequently raised plasma aldosterone concentration; PIND could have blunted these increases. Therefore, the diuretic-induced increase of urinary Mg^{2+} output would have been, at least partly, due to secondary hyperaldosteronism. The potential clinical application of the present findings merit chronic studies in patients suffering from high blood pressure.

Résumé

Cette investigation eut par objetive l'évaluation de l'effet du blocage bêta-adrénergique sur les accroissements des excrétions urinaires de Mg^{2+} et de K^+ induits par les diurétiques.

On administra, de façon double aveugle, des doses uniques de placebo, du diurétique clopamide 5 mg (CLOP), du bêta-bloquant adrénergique pindolol 10 mg (PIND) et de la combination de CLOP et PIND (CLOP + PIND) à neuf volontaires normaux. Chaque dose fut administrée sept jours à part des autres, de manière randomisé. Les sujets furent étudiés chez une salle métabolique, sous des conditions strictement contrôlées.

La CLOP et la CLOP + PIND augmentèrent significativement les moyennes des débits urinaires de 24 heures de liquide et de Na^+ par rapport au placebo. La CLOP augmenta significativement les moyennes des débits urinaires de Mg^{2+} ($\Delta\% = 27$, $p<0{,}01$) et de K^+ ($\Delta\% = 25$, $p<0{,}05$) par relation au placebo, autant que la CLOP + PIND ne modifia pas le débit urinaire d'aucun des deux electrolytes ($\Delta\% = -1$ pour le Mg^{2+} et $\Delta\% = -10$ pour le K^+).

Le PIND pourrait avoir bloqué l'élévation que la CLOP induit sur la production rénale de rénine, et par conséquence le PIND pourrait avoir diminué la concentration plasmatique d'aldostérone. Les accroissements des débits urinaires de Mg^{2+} et de K^+ induits par la CLOP seraient dus, au moins partialement, à l'aldostéronisme secondaire provoqué par ce diurétique. Les avantages potentielles de ces donnés pour le traitment de l'hypertension artérielle devraient être investiguées par des études chroniques.

Introduction

Evidence that essential hypertension constitutes an important cardiovascular risk factor has lead to the recommendation that it should be treated vigorously [44]. Clinical and epidemiological results appear to support this approach [44]. However, in practical terms it is difficult to treat all patients with elevated blood pressure specifically because of a variety of problems, including the high prevalence of hypertension in most modern societies, the fact that the disease is usually asymptomatic and therefore patients are not prone to comply with sophisticated therapeutic regimes, the variability of response to most therapies, the high incidence of significant undesirable side and toxic effects of available drugs and the expense of comprehensive diagnosis and selective management. For these reasons a tendency exists to standardize antihypertensive therapy, in a manner aimed at minimizing the risk/benefit ratio and the costs involved. Initially, success in achieving these goals was limited by the drugs available. The advent of thiazides, some three decades ago, constituted such an important turning-point in this respect that diuretics still remain a cornerstone in the treatment of hypertension [18] and preserve their place as the most commonly prescribed group of drugs for this purpose worldwide [55, 77].

It took some years for the undesirable features of diuretics to become apparent, and to be in-

vestigated in a systematic manner. A policy directed at solving the problems these undesirable effects pose is still under development and, at the same time, new problems are being identified as medicine progresses and the scope and sensitivity of instrumental analysis increase.

The introduction of beta-adrenergic blockers provided an alternative first choice for the drug treatment of essential hypertension, according to some schools [8, 16, 35]. However, these drugs are less efficacious than diuretics in terms of the percentage of cases in which they can normalize blood-pressure values when the unstratified population of patients with essential hypertension is considered. Nevertheless, beta-adrenergic blockers increase the antihypertensive action of diuretics and are consequently often added to diuretic regimens in order to achieve optimal control of blood pressure in as many patients as 82 % [24]. Since beta-adrenergic blockers possess pharmacological properties that affect several systemic variables in an opposite direction to diuretics, the conjoint administration of diuretics and beta-adrenergic blockers has been advocated not only as a means of normalizing blood pressure in a higher number of patients than when either drug is used alone, but also in order to balance out various undesirable pharmacological actions [8, 28].

Common diuretics increase the urinary excretion of K^+ and may thus conduce to somatic depletion of the cation [19, 47]. Diuretic drugs block the reabsorption of filtered Na^+ at the loop of Henle (e.g. furosemide) or at the early portion of the distal tubule (e.g. thiazides and chlorthalidone), thus augmenting the amount of Na^+ available for reabsorption at the terminal portion of the distal convoluted tubule, where it is exchanged for K^+ and H^+ which are excreted into the nephronal lumen. Diuretics activate the renin-angiotensin-aldosterone (RAA) system [10, 12, 19] through the decrease in blood volume and the subsequent increase of beta-1 sympathetic activation they induce [16, 70, 73], and through the decrease in Na^+ concentration in the milieu intérieur they provoke; thus, the urinary excretion of K^+ is elevated since aldosterone augments the exchange between Na^+ and K^+ plus H^+ that takes place in the late distal tubule [12].

Somatic K^+ depletion, secondary to the increase in renal K^+ excretion induced by diuretics, has been widely incriminated as a critical determinant of cardiac arrhythmias [18, 77], myocardial infarction and sudden death which occur in diuretic-controlled hypertensives. Beta-adrenergic blockers, which at variance with diuretics have been labeled as "cardioprotective" [7], counteract diuretic-induced hyperkaliuresis when they are coadministered via the decrease they cause in plasma renin activity, provided the dose relationship between the drugs is correct, as occurs in most fixed-dose combinations which have been developed with this, amongst other, objectives taken into consideration. However, the only major advantage of fixed-dose combinations of diuretics and beta-adrenergic blockers proven to day has been an increased antihypertensive efficacy with respect to either component used alone [6], since combinations effectively control blood pressure in 70–85 % of unstratified cases suffering from essential hypertension. The effects, if any, of these combinations upon the incidences of cardiac arrhythmias, sudden death and myocardial infarction have not been adequately studied. Meanwhile the controverted [40, 46] idea that diuretic-induced K^+ depletion is of central importance to the failure of diuretics to reduce the incidence of important cardiac events in hypertensive patients to the rates found in normotensive individuals, as these drugs reduce the incidences of cardiac insufficiency, renal failure and cerebrovascular accidents [14, 76], has been loosing ground to the concept that diuretic-induced hypermagnesiuresis [3, 4, 27, 36, 52, 54, 56, 75] and resulting somatic Mg^{2+} depletion [26, 29, 30] are at least as important in the overall determination of the mentioned side effects and in the decrease in intracellular K^+ provoked by diuretics [76]. This view, which is based upon clinical and experimental evidence [14, 67, 76], has been the subject of several recent reviews [52, 54, 59, 64].

Somatic Mg^{2+} depletion may occur in many patients during prolonged diuretic therapy, particularly when other factors coexist, nearly all of which are similar to those promoting K^+ deficiency [52, 54]. Most types of diuretics, including the mercurial, the loop (furosemide-like), the early distal tubular (thiazide-like, be them uric-acid retainers or not) and the xantines induce hypermagnesiuresis in normal and diseased man. The subsequent decrease in Mg^{2+} concentration within the myocardial cells results in a depletion of intramyocardial K^+ and in increases in intracellular Na^+ and free cytosolic Ca^{2+} concentrations, this cationic pattern predisposing to serious cardiac arrhythmias and sudden death [22, 52, 54]. A similar ionic shift could occur within vascular muscle during Mg^{2+} depletion leading to vasospasm, principally in the coronary arteries [1]. A positive association has been found between low-Mg^{2+} intake and the risk of developing acute myocardial infarction [39] and sudden death [37]. Both K^+ and Mg^{2+} concentrations are decreased in infarcted myocardium and perinecrotic areas [11]. Patients with severe coronary heart disease, diagnosed by coronary arteriography, have been found to have lower-plasma Mg^{2+} concentration than controls [41]. Magnesium, and to a less extent

K^+, deficiency could be also involved [49, 53] in the mechanism of diuretic-induced decrease in glucose tolerance [17, 53] and in the shift diuretics provoke in the plasma lipid profile towards a pattern associated with an increase in cardiovascular risk (a rise in total and LDL- and VLDL-cholesterol and a fall in HDL-cholesterol [20, 65]). This latter metabolic shift may be counteracted by the coadministration of beta-adrenergic blockers [43]. From these facts it would appear that blunting of diuretic-induced hypermagnesiuresis could be highly beneficial in hypertensive patients. Since plasma Mg^{2+} and renin concentrations are inversely related [50] and since urinary Mg^{2+} excretion seems to be positively influenced by aldosterone in man [21, 42], both ACE-inhibitors and beta-adrenergic blockers could be regarded as likely to provide the counteraction desired. A study by this group has shown that captopril exerts a hypermagnesiuretic effect per se [38]; consequently, the choice of a suitable agent for combination therapy is confined at present to beta-adrenergic blockers.

The objectives of this study were to assess how the acute administration of a combination of a diuretic and a beta-adrenergic blocker to normal subjects affects the urinary outputs of fluid, Mg^{2+}, K^+, Na^+, and other solutes, by comparison with the administrations of placebo and of the active components of the combination given separately.

Subjects and methods

Subjects and experimental design

Nine healthy male adult students volunteered to participate in the study after a full explanation of its implications. All were aged between 18 and 24 and had taken no other medication within the previous 2 months. None was obese nor had any history of renal, cardiovascular, hepatic or metabolic disorders. Smokers or alcoholics were not studied.

A standardized diet containing 200 to 220 mmol of Na^+ and approximately 4000 ml of water was prescribed on treatment days and during the 24 hours preceding each of them (control days). Subjects received placebo, 5 mg clopamide, 10 mg pindolol and a combination of 5 mg clopamide and 10 mg pindolol (CLOP + PIND), separately and in random order, on four different treatment days which were at least 7 days apart. Medications were given at 0800 hour with 100 ml tap water. Volunteers were confined to a metabolic ward on treatment days when ingestion of alcohol and drinks containing caffeine was forbidden. No other medications, including simple analgesics or the topical use of corticosteroids, were allowed during the entire study period.

Each urine specimen collected from 0–3, 3–6, 6–9, 9–12 and 12–24 hours after dosing on treatment days and pooled urine collected during the previous 24 hours were measured for concentrations of Cl^-, Na^+, K^+, Ca^{2+}, Mg^{2+}, inorganic phosphate, Zn^{2+}, creatinine and urate.

On treatment days, blood was drawn by venipuncture just before medication and from 5.5 to 6.5 and 23.5 to 24.5 hours later for measurement of serum concentrations of Cl^-, Na^+, K^+, Ca^{2+}, Mg^{2+}, inorganic phosphate, creatinine, blood urea nitrogen (B. U. N.), urate, total CO_2 and glucose. Blood was collected in plain glass tubes and serum was separated by centrifugation and frozen until chemical analysis.

Laboratory methods

All laboratory analyses were carried out by technicians who were unaware of the protocol.

Urinary and serum variable concentrations were measured as follows: Na^+ and K^+ were evaluated with an IL 943 flame photometer using caesium as diluent. Cl^- was measured with an IL 446 analyser using a mercuric diluent. Total CO_2 was determined with the same instrument by conversion of bicarbonate into CO_2 by acid treatment and measurement of pCO_2 by means of a glass pH electrode/CO_2 permeable chamber. Magnesium, Ca^{2+} and Zn^{2+} were measured by atomic absorption with a Varian 1275 instrument. Inorganic phosphate, creatinine, urate, B.U.N. and glucose were all determined colorimetrically on a Model 34 Beckman Trace II spectrophotometer. Colorimetric reactions were used for the assays of total inorganic phosphate (Clinical Sciences kit) and creatinine (Boehringer-Mannheim kit); enzymatic colorimetric reactions were employed for the evaluations of uric acid (Boehringer-Mannheim Peridochrom kit), B.U.N. and glucose (Beckman kits).

Mathematical methods

All experimental values are expressed as means ± S.E.M. The mean experimental values of the urinary volume and solutes, accumulated by the end of each post-dosing collecting period on treatment days, M, as functions of time, t, were fitted by a mathematical model [51]:

$$20M/\log(100-M) = \exp[2.30\,(t-t_l)/(a+bt)],$$

where t_l is the time at which M = 0.1 unit used for the fitting and a (time) and b (dimensionless) are the zero ordinate value and the slope parameters of the regression of the linearised transformation of the function respectively. The fitting procedure was described previously [51].

Flows of urinary variables were defined as the derivative of M with respect to time [51]:

$$dM/dt = (a+bt_l)/[(a+bt)^2)\{(0.43/M)+(0.43^2/[(100-M)\log(100-M)]\}].$$

This derivation is graphically exemplified in Fig. 1. Flows were characterized by the times to

peak flows of urinary variables after dosing, t_m, since a nihil derivative of the latter function with respect to time ($d^2M/dt^2 = 0$) exists when flow is maximal, and t_m summarizes the parametric relationships between t_l, a and b in the analytical expression for d^2M/dt^2. Mean time to peak flow of each urinary variable after dosing was calculated on a computer, from the corresponding flow function, through an iterative procedure.

Normality of frequency distributions and homoscedasticity of sample variances were evaluated, for all variables, through the chi-square test and the F ratio respectively. Since only minor departures from formal prerequisites for parametric statistical-

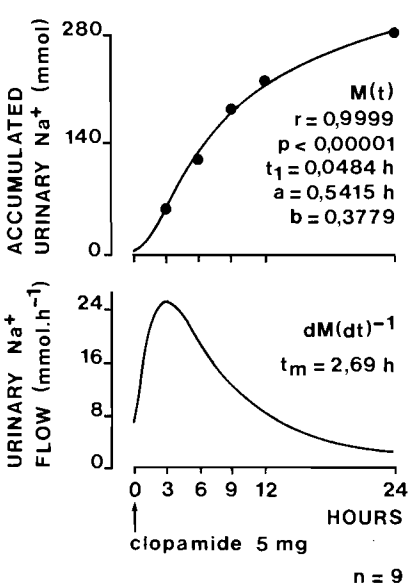

Fig. 1: Top: mean accumulated urinary Na^+ output (dots) after administration of a single dose of clopamide 5 mg per os to nine healthy volunteers at time 0 (0800 h), M(t) is the *Reyes* and *Leary* mathematical model [51] (continuous-function curve), that has been fitted to the experimental means. t_l, a and b are the parameters of the function.
Bottom: mean urinary Na^+ flow after administration of a single dose of clopamide 5 mg per os to nine healthy volunteers at time 0 (0800 h). The graph has been evaluated as the derivative of the function in the top panel with respect to time (dM/dt). Any area between the curve and the abscissae axis and between any two times represents the amount of electrolyte excreted between the times that constitute the area limits. t_m is the time from dosing to maximal flow.

techniques were detected in a few cases, all descriptive and inferential methods used were parametric. Correlation and regression on linearised data and the paired t-test were deployed. All statistical tests were two-tailed and $p = 0.05$ was considered the limit of significance.

Results

No significant differences were found between the mean 24-h accumulated urinary outputs of fluid and solutes after placebo and those yielded on control days. The urinary outputs of Cl^-, Na^+, fluid, K^+, Ca^{2+}, Mg^{2+}, inorganic phosphate, Zn^{2+}, creatinine and urate accumulated at 3, 6, 9, 12 and 24 h after the intake of placebo, clopamide 5 mg, pindolol 10 mg and of CLOP + PIND are shown in Table I.

The significances of the differences between the mean 24-h urinary outputs after placebo and after the active medications are presented in Table II. Clopamide 5 mg significantly increased the mean urinary outputs of Cl^-, Na^+, fluid, K^+, Mg^{2+}, and significantly decreased those of creatinine and urate with respect to placebo, while leaving the mean 24-h outputs of Ca^{2+}, inorganic phosphate and Zn^{2+} unchanged. CLOP + PIND significantly increased the mean 24-h urinary outputs of Cl^-, Na^+ and fluid with respect to placebo, left those of K^+, Mg^{2+}, Ca^{2+}, inorganic phosphate, Zn^{2+} and creatinine unchanged and significantly decreased the mean urinary output of urate. Pindolol 10 mg did not affect any of the mean 24-h outputs studied to a statistically significant degree. When the effects of clopamide 5 mg on the mean 24-h urinary outputs of fluid and solutes were compared to those of CLOP + PIND, the only significant difference found consisted in a reduction in kaliuresis by the combination with respect to clopamide alone (Table II). When the effects of pindolol 10 mg were compared to those of CLOP + PIND, significant increases in mean 24-h urinary outputs of Cl^-, Na^+ and fluid in response to CLOP + PIND were detected, whereas no significant differences existed between outputs of the other variables. Clopamide 5 mg induced significantly higher mean 24-h urinary outputs of Cl^-, Na^+, fluid, K^+, Mg^{2+} and Zn^{2+} than pindolol 10 mg, whilst no significant differences arose for Ca^{2+}, inorganic phosphate, creatinine and urate. Figure 2 shows the percentual changes in the mean 24-h urinary outputs of Na^+, K^+ and Mg^{2+} with respect to corresponding outputs after placebo. It appears clear that clopamide 5 mg and CLOP + PIND exerted impressive natriuretic effects of similar magnitude whilst pindolol 10 mg did not affect mean 24-h urinary Na^+ output. It is also evident that whereas clopamide 5 mg significantly increased kaliuresis and magnesiuresis with respect to placebo, pindolol 10 mg caused urinary retentions of both cations which, although statistically non-significant with respect to placebo, counteracted the effects of clopamide on both variables as revealed by the absence of any statistically significant action of CLOP + PIND on K^+ and Mg^{2+} outputs with respect to placebo.

The mathematical model [51] used for the accumulated outputs of fluid and solutes fitted the data satisfactorily in all cases, thus permitting evaluation of both the corresponding mean flows as functions of time and the times to peak flows after dosing. Table III shows the statistical features of the linearized versions of the M(t) functions and their parameter values.

The mean urinary flows of Mg^{2+}, Ca^{2+}, Na^+ and K^+ after placebo and after clopamide

5 mg are illustrated in Fig. 3. The relationships between the time courses of the post-placebo and post-clopamide flows were homomorphic for Mg^{2+} and Ca^{2+}, i.e. an initial increase in flows caused by clopamide was followed by a small decrease (undershoot) with respect to the corresponding placebo response. In the case of Na^+ and K^+ the relationships between the post-placebo and post-clopamide flows were heteromorphic between the two ions and show a different pattern from that of the Mg^{2+} and Ca^{2+} flow relationships (Fig. 3).

Figure 4 reveals that the mean 24-h urinary flows of Mg^{2+} and K^+ after placebo and pindolol 10 mg were homomorphic; an initial phase where the post-pindolol flows had higher values than their post-placebo counterparts was succeeded by a period where the reverse occurred. The relationships between the post-placebo and post-pindolol flows of Ca^{2+} and of Na^+ exhibited different patterns for each of the cations and were also at variance with reference to the Mg^{2+} and K^+-flow-relationship pattern.

The relationships between post-placebo and post-CLOP + PIND mean urinary flows of Mg^{2+}, Ca^{2+}, Na^+ and K^+ are depicted in Fig. 5. The Mg^{2+}, Ca^{2+} and K^+ flows were homomorphic in the sense that a primary phase, when the post-

Table I: Accumulated excretions of urinary variables after administration of single doses of clopamide 5 mg, a combination of clopamide 5 mg and pindolol 10 mg (CLOP + PIND) and pindolol 10 mg to nine healthy volunteers. Values as mean ± S.E.M.

Urinary variable	Medication	Hours after dosing				
		3*	6	9	12	24
Chloride (mmol)	Placebo*	35±8	66±13	93±17	126±21	190±31
	Clopamide 5 mg	64±9	142±14	229±21	272±23	349±26
	CLOP + PIND	65±6	152±15	230±21	273±24	364±23
	Pindolol 10 mg	26±4	63±9	97±11	120±12	174±15
Sodium (mmol)	Placebo	25±6	52±10	75±13	101±16	154±23
	Clopamide 5 mg	56±7	119±12	188±21	220±22	285±26
	CLOP + PIND	57±4	122±13	186±20	224±24	307±24
	Pindolol 10 mg	17±3	43±8	73±11	96±13	148±12
Fluid (litre)	Placebo	0.48±0.09	1.10±0.12	1.55±0.15	2.00±0.15	3.11±0.18
	Clopamide 5 mg	0.60±0.10	1.35±0.11	2.26±0.13	2.76±0.13	3.90±0.10
	CLOP + PIND	0.78±0.07	1.72±0.16	2.51±0.23	2.98±0.21	4.00±0.19
	Pindolol 10 mg	0.57±0.09	1.35±0.10	1.93±0.13	2.42±0.12	3.25±0.10
Potassium (mmol)	Placebo	13.3±1.7	26.4±2.3	32.9±2.7	41.8±3.1	61.3±5.0
	Clopamide 5 mg	15.6±2.3	30.9±2.4	46.2±3.1	55.3±3.7	76.9±5.8
	CLOP + PIND	14.4±1.8	25.5±2.8	34.4±3.4	39.9±4.3	54.9±4.3
	Pindolol 10 mg	11.4±1.2	22.6±2.3	30.0±2.9	36.9±2.5	49.0±2.1
Calcium (mmol)	Placebo	0.56±0.07	1.08±0.11	1.69±0.17	2.22±0.25	4.80±0.53
	Clopamide 5 mg	0.91±0.11	1.74±0.22	2.61±0.37	3.12±0.46	4.94±0.73
	CLOP + PIND	0.93±0.16	1.88±0.26	2.49±0.33	2.86±0.38	4.21±0.66
	Pindolol 10 mg	0.53±0.08	1.34±0.17	1.92±0.19	2.79±0.34	4.90±0.74
Magnesium (mmol)	Placebo	0.53±0.07	1.14±0.08	1.72±0.13	2.17±0.19	4.97±0.40
	Clopamide 5 mg	0.96±0.05	1.80±0.11	2.81±0.24	3.36±0.32	6.32±0.62
	CLOP + PIND	0.87±0.09	1.80±0.14	2.44±0.23	3.02±0.37	4.90±0.88
	Pindolol 10 mg	0.53±0.05	1.27±0.09	1.76±0.20	2.40±0.30	4.25±0.54
Inorganic phosphate (mmol)	Placebo	5.1±1.1	8.4±1.3	13.0±1.6	19.1±1.8	42.8±3.5
	Clopamide 5 mg	4.1±1.4	6.3±1.6	12.3±2.2	16.5±2.6	36.9±4.0
	CLOP + PIND	3.0±0.5	7.8±1.7	13.4±2.1	20.4±3.1	41.1±2.5
	Pindolol 10 mg	2.2±0.6	4.9±0.7	9.1±1.2	15.9±2.3	35.6±2.9
Zinc (μmol)	Placebo	1.17±0.28	1.92±0.39	2.90±0.52	3.79±0.66	8.11±1.23
	Clopamide 5 mg	1.80±0.28	3.04±0.39	4.92±0.70	5.98±0.89	10.16±1.67
	CLOP + PIND	1.81±0.31	3.07±0.50	4.59±0.80	5.40±1.01	8.56±1.74
	Pindolol 10 mg	1.19±0.18	2.16±0.31	3.23±0.53	4.08±0.57	6.60±0.75
Creatinine (μmol)	Placebo	3.5±0.3	6.0±0.4	8.4±0.6	11.4±0.7	22.0±1.5
	Clopamide 5 mg	3.4±0.3	5.7±0.5	9.0±0.7	11.3±0.8	20.6±1.2
	CLOP + PIND	3.6±0.2	6.6±0.7	9.7±0.8	12.1±1.0	22.0±1.1
	Pindolol 10 mg	2.8±0.2	5.5±0.4	8.3±0.6	11.2±0.7	20.4±0.9
Urate (mmol)	Placebo	1.18±0.24	1.95±0.23	2.78±0.28	3.72±0.39	6.35±0.59
	Clopamide 5 mg	0.93±0.11	1.58±0.18	2.72±0.30	3.35±0.38	5.39±0.52
	CLOP + PIND	0.84±0.08	1.56±0.18	2.48±0.28	3.10±0.34	4.99±0.40
	Pindolol 10 mg	0.68±0.05	1.36±0.11	2.26±0.19	3.07±0.24	5.08±0.23

* Hour-3 placebo values were derived from seven cases.

CLOP + PIND flow surpassed the post-placebo flow, was followed by an undershoot of the post-active-medication flow with respect to that after placebo. At variance, the post-CLOP + PIND mean urinary Na^+ flow was above its post-placebo counterpart throughout the 24-hour period.

Figure 6 shows that the relationships between post-clopamide and post-CLOP + PIND mean urinary flows of Mg^{2+}, Ca^{2+} and K^+ were homomorphic; a first phase during which the post-CLOP + PIND flow surpassed the post-clopamide flow was followed by a period when the reverse took place. Urinary flow of Na^+ after CLOP + PIND did not follow this pattern but was above the corresponding post-pindolol flow throughout the 24-h period.

Mean values of serum variables before and after dosing with the four formulations are shown in Table IV. Statistically significant falls in serum K^+ and elevations in serum urate level were noted 6 and 24 hours after dosing with CLOP + PIND and 24 hours after clopamide 5 mg and pindolol 10 mg. Mean serum K^+ concentration also rose significantly 24 hours after placebo. B.U.N. fell 6 hours after all formulations and rose 24 hours after placebo and clopamide. Total CO_2 was significantly increased 24 hours after clopamide. Serum Ca^{2+} level was reduced 24 hours after placebo. Serum glucose rose 24 hours after CLOP + PIND, and mean serum inorganic phosphate was increased 6 hours after placebo.

Discussion

Urinary outputs of fluid and electrolytes

The effect that clopamide 5 mg had in increasing the mean 24-h urinary outputs of Cl^-, Na^+, fluid, K^+ and Mg^{2+} with respect to placebo and the decreases it induced in the outputs of creatinine and urate could be expected on the basis of current knowledge about the actions of diuretics with renal acceptors principally situated in the first portion of the distal convoluted

Table II: Probabilities corresponding to dependent-t statistics evaluating the significance of the differences between any two mean 24-hour urinary variable outputs after administration of single doses of clopamide 5 mg, a combination of clopamide 5 mg and pindolol 10 mg (CLOP + PIND) and pindolol 10 mg.

Urinary variable	Medication	Medication		
		Placebo	Pindolol 10 mg	Clopamide 5 mg + pindolol 10 mg
Chloride	Clopamide 5 mg	0.0025*	<0.0001*	0.5796
	CLOP + PIND	<0.0001*	<0.0001*	
	Pindolol 10 mg	0.5902		
Sodium	Clopamide 5 mg	<0.0001*	0.0003*	0.4464
	CLOP + PIND	<0.0001*	<0.0001*	
	Pindolol 10 mg	0.7099		
Fluid	Clopamide 5 mg	0.0003*	0.0001*	0.6002
	CLOP + PIND	0.0012*	0.0019*	
	Pindolol 10 mg	0.2240		
Potassium	Clopamide 5 mg	0.0291*	0.0010*	0.0006*
	CLOP + PIND	0.3617	0.1490	
	Pindolol 10 mg	0.0629		
Calcium	Clopamide 5 mg	0.7911	0.9208	0.2979
	CLOP + PIND	0.3362	0.4306	
	Pindolol 10 mg	0.8524		
Magnesium	Clopamide 5 mg	0.0091*	0.0080*	0.1249
	CLOP + PIND	0.9362	0.4215	
	Pindolol 10 mg	0.1953		
Inorganic phosphate	Clopamide 5 mg	0.1194	0.7004	0.3323
	CLOP + PIND	0.4395	0.1265	
	Pindolol 10 mg	0.0965		
Zinc	Clopamide 5 mg	0.0901	0.0124*	0.1567
	CLOP + PIND	0.7750	0.1827	
	Pindolol 10 mg	0.2053		
Creatinine	Clopamide 5 mg	0.0256*	0.8123	0.2135
	CLOP + PIND	0.9679	0.2547	
	Pindolol 10 mg	0.1325		
Urate	Clopamide 5 mg	0.0382*	0.5376	0.3294
	CLOP + PIND	0.0179*	0.7940	
	Pindolol 10 mg	0.0639		

* Significant.

tubule. Zinc urinary output rose and inorganic phosphate urinary output decreased after clopamide administration, but these changes were not statistically significant with respect to placebo, although the probabilities of confirmation of the null hypothesis were borderline with those of rejection in each case (Table II); the relationship between the small sample size and the arbitrary level of significance set could account for these findings. The mean urinary Ca^{2+} output after clopamide did not differ from that after placebo; this is not at variance with the Ca^{2+}-retaining effect expected of an early distal tubular diuretic like clopamide [5], since it usually only becomes apparent after repeated administration of the diuretic.

Pindolol did not affect the mean 24-h urinary outputs of any variable to a statistically significant degree, although the decreases in the outputs of K^+ and inorganic phosphate (Table I) after pindolol 10 mg with respect to post-placebo excretions were near the border of significance (Table II), and would become significant upon an increase in the alpha level set and possibly also if the sample analysed were larger. When directionally compared with the outputs which followed clopamide administration, the post-pindolol urinary outputs showed opposite tendencies for Cl^-, Na^+, fluid, K^+, Mg^{2+} and Zn^{2+} (Table I); these contrary actions achieved statistical significance.

Beta-adrenergic blockers induce K^+-retention through an inhibition of renal renin release, mainly due to beta-1-adrenergic blockade in man [66], and secondary decreases in angiotensin-II formation and plasma aldosterone concentration. The action of

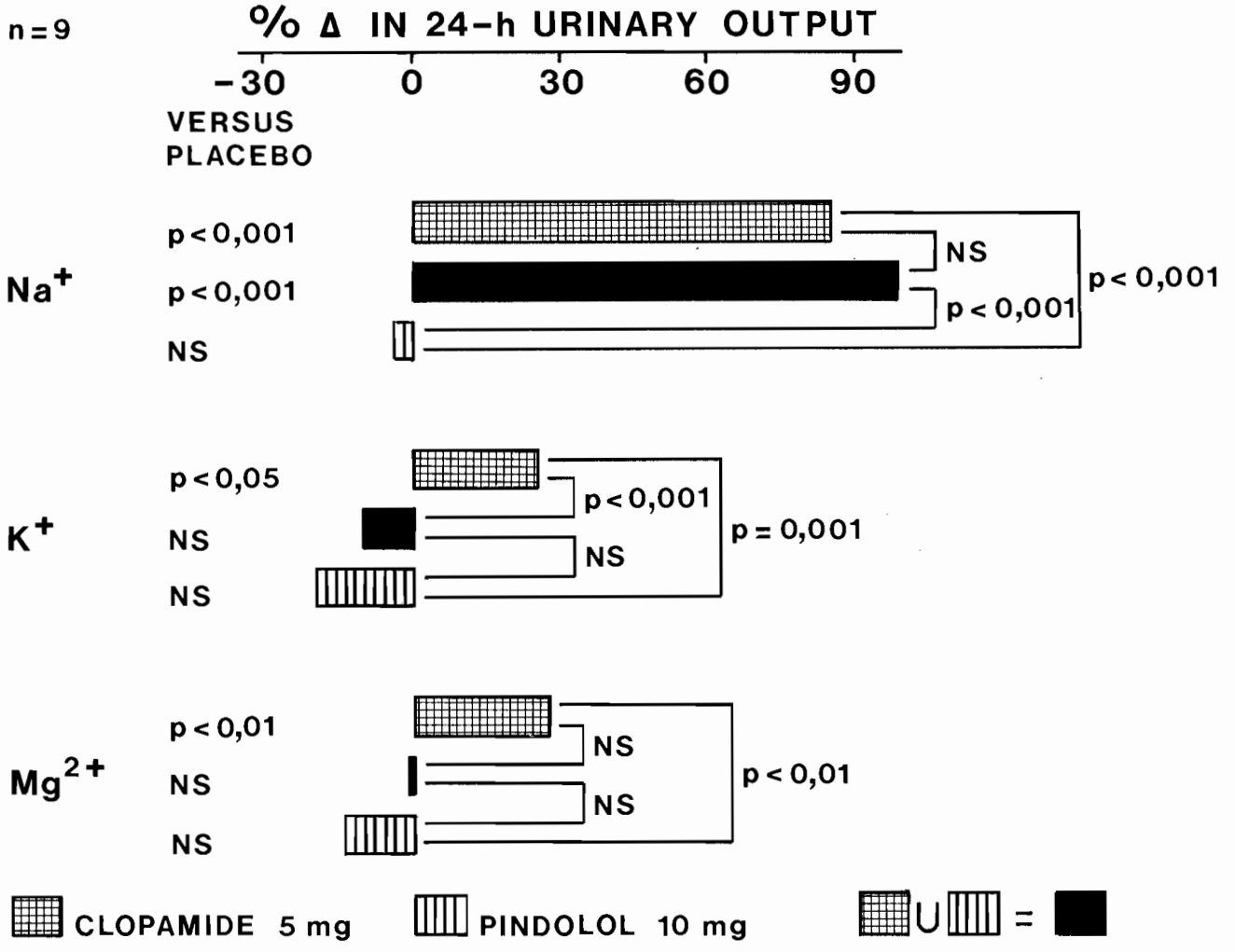

Fig. 2: Percentage changes in the mean 24-h urinary outputs of Na^+, K^+ and Mg^{2+} after separate administration of single doses of clopamide 5 mg, a combination of clopamide 5 mg and pindolol 10 mg, and pindolol 10 mg per os to nine healthy volunteers. The probability values refer to comparisons between corresponding mean values.

pindolol, which exhibits higher intrinsic sympathomimetic activity (ISA) than most current beta-adrenergic blockers [66], on the RAA system has been the subject of much controversy, apparently derived from different experimental designs and results. Consistent reports exist, however, indicating that pindolol decreases the activity of the RAA system when it has previously been activated [2, 78], or when the coadministration of a diuretic tends to enhance this activity [65]. A result of this interaction was observed in the present study when CLOP + PIND did not affect the urinary K^+ output, despite of having significantly increased the renal urinary output of Na^+ and therefore the availability of this cation at the late distal convoluted tubule where Na^+ is exchanged for K^+ and H^+. Pindolol 10 mg per se did not decrease the mean 24-h urinary output of Na^+ with respect to placebo, which could mean plasma aldosterone concentration was reduced, to the necessary extent for this effect, by the beta-adrenergic blocker [27]. CLOP + PIND acted on Cl^-, Na^+ and fluid outputs to the same extent as clopamide 5 mg (Table II). The lack of any detrimental effect of pindolol on natriuresis, when added to the diuretic, suggests that the beta-adrenergic blocker did not promote further Na^+ excretion despite counteracting the diuretic-induced enhancement of the RAA system; this could be due to an haemodynamic effect of pindolol

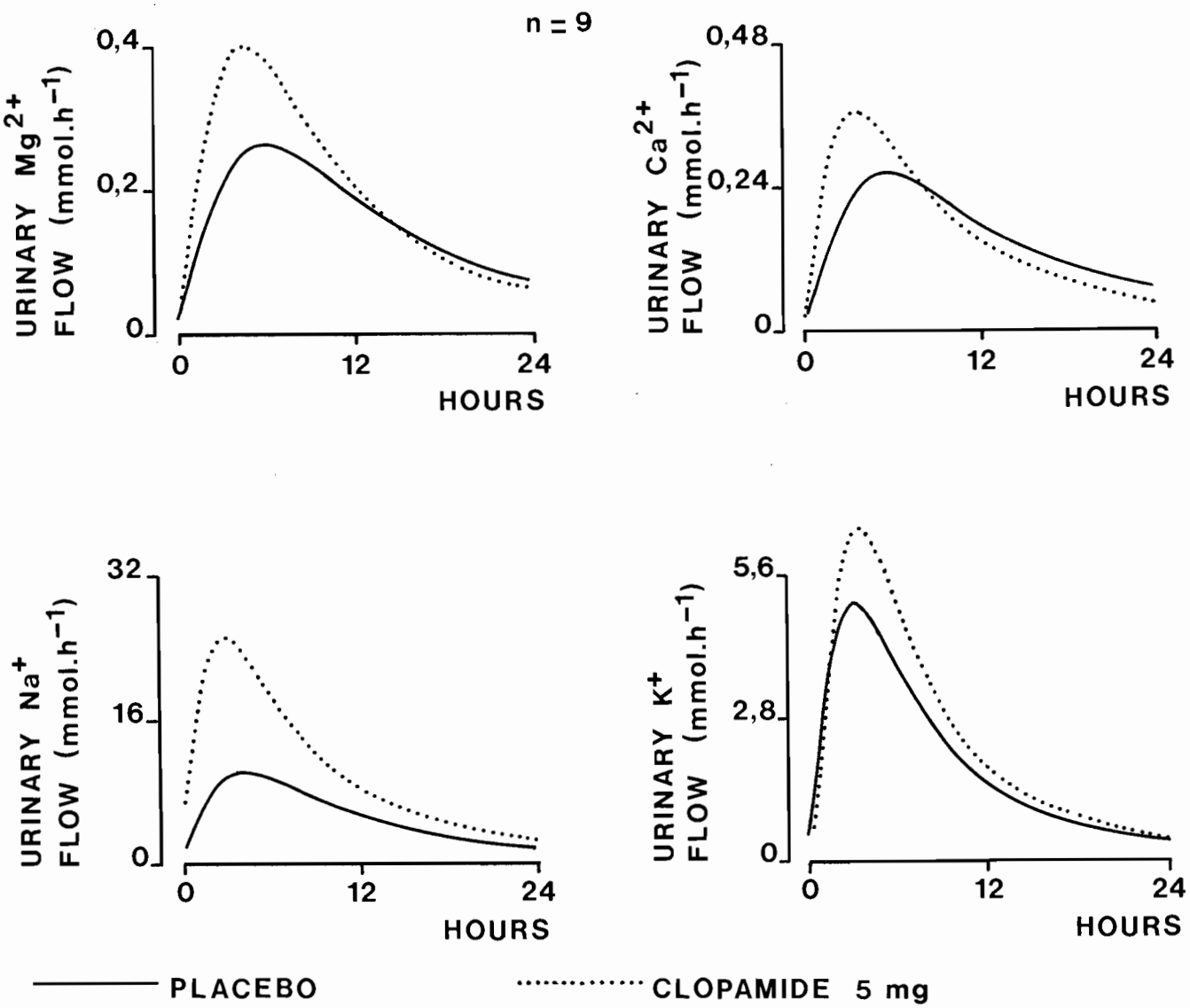

Fig. 3: Mean urinary Mg^{2+}, Ca^{2+}, Na^+ and K^+ flows after separate administration of placebo and of a single dose of clopamide 5 mg per os to nine healthy volunteers at time 0 (0800 h). The relationship between the post-placebo and post-clopamide flows of Mg^{2+} is homomorphic with respect to the relationship between the post-placebo and post-clopamide flows of Ca^{2+}, i.e. each of these two relationships shows two analogous phases. The relationship between the post-placebo and post-clopamide flows of Na^+ and that corresponding to K^+ exhibit different patterns with respect to each other and are also at variance with the flow-relationship pattern common to Mg^{2+} and Ca^{2+}.

(vasodilation) that would tend to cause retention of Na^+ via a reflex increase in plasma aldosterone. This potential Na^+-retaining property of pindolol was not exhibited by the drug alone, but could have become overt when beta-adrenergic blockade counteracted the enhanced activity of the RAA system induced by clopamide 5 mg; the resultant fall in the serum level of angiotensin II, which has a permissive action on the effects of adrenergic stimulation, would have counteracted the intrinsic sympathomimetic activity of pindolol.

CLOP + PIND did not affect the 24-h urinary output of Mg^{2+} significantly when compared to placebo. The role of aldosterone in the control of renal Mg^{2+} handling has been the subject of much debate, mainly on the basis of animal experiments [42]. However, there is evidence that aldosterone increases renal Mg^{2+} excretion and subsequently reduces plasma Mg^{2+} concentration in man [23], although this does not appear to be the principal regulatory mechanism of the renal excretion of the cation, at least under physiological circumstances. A dissimilitude in the relative importances of the RAA system in the renal handlings of K^+ and Mg^{2+}, when the system is activated by a diuretic, is shown by the fact that the differences between the mean urinary outputs of K^+ after clopamide 5 mg and after CLOP + PIND was highly significant statistically (Table II), whereas the

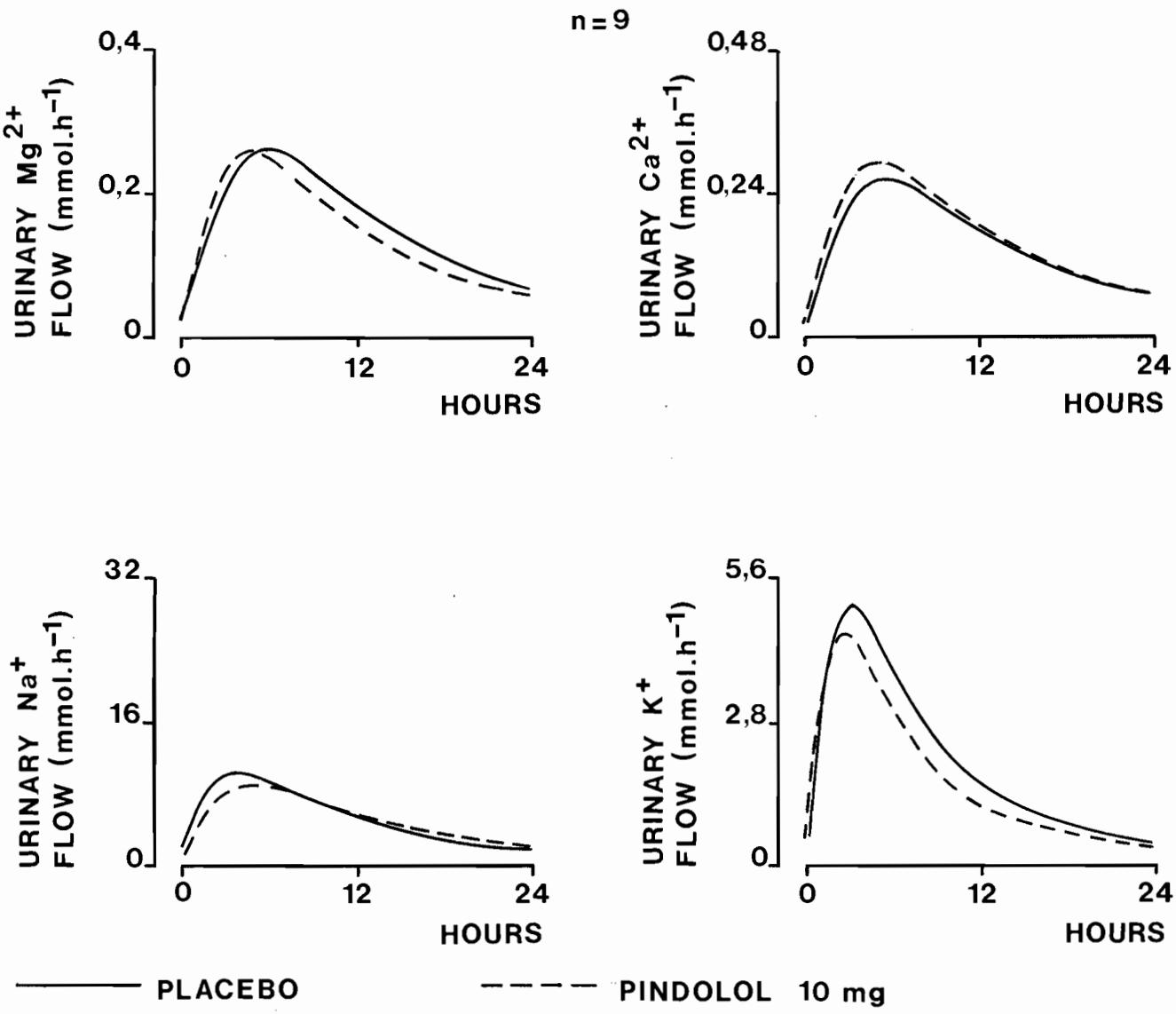

Fig. 4: Mean urinary Mg^{2+}, Ca^{2+}, Na^+ and K^+ flows after separate administration of placebo and of a single dose of pindolol 10 mg per os to nine healthy volunteers at time 0 (0800 h). The relationship between the post-placebo and post-pindolol flows of Mg^{2+} is homomorphic with respect to the relationship between the post-placebo and post-pindolol flows of K^+, i.e. each of these two relationships shows two analogous phases. The relationship between the post-placebo and post-pindolol flows of Ca^{2+} and that corresponding to Na^+ exhibit different patterns with respect to each other and are also at variance with the flow-relationship pattern common to Mg^{2+} and K^+.

corresponding difference in mean urinary Mg^{2+} output did not attain statistical significance, although it approached it (Table II). In addition, despite the fact that both differences lay outside the significant range, the difference between the K^+ outputs after pindolol 10 mg and after CLOP + PIND had a lower probability of rejection of the null hypothesis than its Mg^{2+} counterpart, which would also support the postulate that aldosterone is a more important regulator of renal K^+ than of renal Mg^{2+} excretion, at least when a common early distal convoluted tubular diuretic is administered.

The opposite, albeit statistically non-significant, effects of clopamide 5 mg and pindolol 10 mg on urinary Zn^{2+} output with respect to placebo became statistically significant when the effects of the active drugs were directly compared (Table II). This fact, which would seem to indicate beta-adrenergic blockade opposes renal Zn^{2+} excretion, is difficult to interpret in detail since knowledge of the renal handling of Zn^{2+} is limited [61, 62].

The facts clopamide and CLOP + PIND caused significant increases in the mean 24-h urinary output of urate with respect to placebo, and that the corresponding effect of pindolol was directionally similar and nearly reached significance, could be expected on the basis of established knowledge that early distal tubular diuretics and beta-adrenergic blockers diminish renal urate excretion.

Urinary flows of fluid and solutes

The mathematical model used [51] fitted the outputs of urinary variables accumulated as functions of time in a highly satisfactory manner (Table III). The validity of the application of the model and the accuracy with which it describes the urinary excretion of any natural or exogenous solute has been discussed elsewhere [51]. Since the model held in the case of all variables after all formulations, the time courses of the mean flows, which were described in terms of the derivative of the fitted function with respect to time, constitute reliable descriptions. The times to maximal flows after dosing, t_m, presented in Table III afford a variable for the simplified overall description of the time courses of all the mean fluid and solute flows.

The directional effect of clopamide on the mean urinary flows of Cl^-, Na^+, Ca^{2+}, Mg^{2+} and Zn^{2+} consisted in an acceleration, whereas the mean flows of fluid, K^+, inorganic phosphate, creatinine and urate were practically unaffected (Table III). These facts indicate that urinary Cl^- and Na^+ excretions were strongly linked, whereas that of fluid could be more related to the changes in urine osmolality that certainly followed the administration of the diuretic. In this particular case, the excretion of K^+ appears to be related to that of Na^+ in a looser manner than usual after the administration of diuretics; this fact, which confirms clopamide is pharmacologically different from the thiazides [45, 72], is perhaps due to that clopamide acts from both the tubular lumen and the milieu intérieur, whereas the thiazides only act from the tubular lumen [45].

The reductions in the t_m values of Na^+ and Mg^{2+} induced by clopamide with respect to placebo were 1.25 and 1.22 hours respectively (Table III), a finding at variance with the most frequently observed response to loop and early distal tubular diuretics, which is a widening of the gap between t_m values of Na^+ and Mg^{2+} after administration of the drug. This result is consistent with the lack of any acute effect by clopamide 5 mg on the t_m of K^+, indicating an association between the mechanisms that account for Mg^{2+} and K^+ excretions after clopamide, which cannot be inferred from any studies involving acidic thiazide-type diuretics acting solely from within the tubular lumen.

With respect to placebo, CLOP + PIND had similar directional effects to clopamide 5 mg on the t_m values corresponding to Cl^-, Na^+, Ca^{2+}, Mg^{2+}, Zn^{2+} and creatinine (Table III). The differences between the post-placebo and post-CLOP + PIND t_m values for Cl^-, Na^+ and Zn^{2+} were lower than the corresponding differences between the post-placebo and post-clopamide 5 mg t_m values and higher than the differences corresponding to Ca^{2+} and Mg^{2+}. CLOP + PIND accelerated the flows of creatinine, fluid and K^+, which had not been affected by clopamide 5 mg, and delayed the inorganic phosphate flow with respect to placebo. These changes are difficult to analyse on the sole basis of the t_m values and are best understood by examining the entire time courses of the flows.

Figure 3 shows the mean urinary flows of Mg^{2+}, Ca^{2+}, Na^+ and K^+ during the 24-h periods following the administrations of placebo and clopamide 5 mg. The diuretic caused initial increases in the mean flows of Mg^{2+} and Ca^{2+} with respect to placebo, corresponding to augmentations of the outputs of these cations. This phase ended at a point at which the mean flows after clopamide turned to fall below those of placebo until the completion of the 24-h post-dosing period. This decrease, which may be referred to as an undershoot of urinary flow since it follows an initial increase in the output of an urinary variable caused by a formulation with respect to placebo, is also apparent from the data in Table I. When an undershoot is much below its reference mean flow or is very prolonged, as occurred with Ca^{2} ^{N} (Fig. 3), the outcome may be a balance between overshoot and undershoot that almost equalises the flows being compared during the 24-h period in question, thus

accounting for the lack of statistically significant changes in the 24-h outputs of the variable concerned. The undershoots could be determined by either a diminution of the source of the solute (decrease in its plasma concentration) or by the activation of regulatory mechanisms tending to conserve the solute in question. In the case of Ca^{2+}, the initial increase in the flow and output of the variable in urine was accompanied by an increase in plasma Ca^{2+} concentration, which in spite of being a non-significant change could be relevant in so far as there was a directionally opposite change (non-significant decrease) in plasma Ca^{2+} 6 hours after the administration of placebo with respect to the pre-dosing mean concentration of the variable (Table IV). This constancy in plasma Ca^{2+} concentration after the diuretic might well have been the result of an elevation in serum parathyroid hormone (PTH), secondary to the increase in calciuresis recorded from 0 to 3 and from 3 to 6 hours after dosing with clopamide 5 mg, which could have determined a fall in plasma Ca^{2+} concentration earlier than 6 hours after dosing thus raising plasma PTH; the increase in plasma PTH could in turn have caused an increase in Ca^{2+} and Mg^{2+} reabsorptions in the loop of Henle with a corresponding decrease in the urinary excretions of Mg^{2+} and Ca^{2+} consistent with the undershoots observed. The effect of PTH on the reabsorption of filtered Mg^{2+} at the loop of Henle would not appear to be as important as it is for Ca^{2+}, since the magnitude of the undershoot in mean urinary Mg^{2+} flow after clopamide was lower than that of mean urinary Ca^{2+} flow, to the extent that the Mg^{2+} undershoot did not balance out the effect of the previous increase in mean urinary flow on Mg^{2+} output, as occurred with respect to Ca^{2+}. This was perhaps due to the fact PTH promotes renin secretion [69], and subsequently elevates urinary Mg^{2+} excretion. The homomorphism of the relationships between the post-placebo and post-clopamide urinary Mg^{2+} and Ca^{2+} flows (both consist of an overshoot and an undershoot phases) would be principally explained by related underlying mechanisms affecting urinary Ca^{2+} and Mg^{2+} excretions. If urinary Ca^{2+} output were reduced when clopamide is chronically administered, as it happens with the other early distal tubular diuretics, plasma PTH would be consequently decreased [68], thus conducing to further urinary Mg^{2+} losses by a reduction in PTH-dependent Mg^{2+} reabsorption in the loop of Henle [52, 54].

The lower panel of Fig. 3 shows that the mean urinary Na^+ flow after clopamide was higher than the mean post-placebo flow over the entire study period of 24 hours, and that a similar consideration holds, in practical terms, for the mean K^+ flow. Since increases in Na^+ excretion induced by diuretics entail a rise in the amount of the electrolyte available for K^+ and H^+ exchange in the last portion of the distal tubule, the homomorphism between urinary K^+ and Na^+ flows after the administration of clopamide and placebo would indicate a related underlying mechanism of a different nature to that accounting for Mg^{2+} and Ca^{2+} flows. The exchange between Na^+ and K^+ in the distal tubule is augmented by the secondary hyperaldosteronism caused by the increase in urinary Na^+ output when a diuretic is administered. Since this effect is known to become physiologically important within a few hours of the commencement of the natriuretic action of a diuretic and to extend for some hours, it could also partly explain the relatively unimpressive undershoot in urinary Mg^{2+} flow following clopamide 5 mg. Further evidence for an aldosterone-mediated contribution to clopamide-induced hyperkaliuresis and hypermagnesiuresis is provided by the fact that the difference between mean urinary K^+ flows after placebo and clopamide was lower than that between the Na^+ flows. More evidence on the importance of aldosterone in urinary Mg^{2+} excretion might be afforded by similar experiments in which Na^+ intake were manipulated.

The time courses of the mean urinary flows of Mg^{2+}, Ca^{2+}, Na^+ and K^+ that followed the administrations of placebo and pindolol 10 mg are comparatively shown in Fig. 4. Beta-adrenergic blockers could affect the active physio-biochemical mechanisms regulating the excretions of Mg^{2+} and Ca^{2+} at the parathyroid gland and renal levels, by reducing the activity of adenylate cyclase, which is under the positive control of catecholamines, and therefore the formation of cyclic adenosine monophosphate (cAMP) [52, 54]. Beta-adrenergic blockers could also affect the processes underlying urinary Na^+ and K^+ excretions by influencing plasma aldosterone levels, since the release of renin by the kidney is under the positive control of the sympathetic system at nephronal beta receptors and most aldosterone secretion is angiotensin-II dependent. However, in the particular case of pindolol, the fact it possesses marked ISA precludes any clear analysis of the flows shown in Fig. 4 because, in addition to uncertainty regarding the effects of ISA in this situation, the blocking action of pindolol of the RAA system may only be unequivocally assumed to exist when the system is activated concomitantly with the administration of pindolol.

The responses of the mean urinary flows of Mg^{2+}, Ca^{2+}, Na^+ and K^+ to the administrations of placebo and CLOP + PIND are shown in Fig. 5, where the flow

relationships for Mg^{2+}, Ca^{2+} and K^+ are clearly homomorphic, whilst the urinary Na^+ flow relationship is an outlier. In the cases of Mg^{2+}, Ca^{2+} and K^+, an initial increase in mean urinary flow after CLOP + PIND with respect to placebo was followed by an undershoot. The blunting of aldosterone production by pindolol would account for the marked undershoot of urinary Mg^{2+} flow following CLOP + PIND with respect to placebo. Similarly, a pindolol-induced decrease in aldosterone would account for the undershoot in K^+ flow with respect to placebo following CLOP + PIND administration; thus, the homomorphism between the post-CLOP + PIND and post-placebo flows of Mg^{2+} and K^+ would be similarly explained by the fact that the participation of aldosterone in the regulation of renal K^+ and Mg^{2+} flows became more evident when the RAA system was activated by clopamide and simultaneously inhibited by pindolol. The homomorphism of the relationship between the post-placebo and post-CLOP + PIND mean urinary flows of Mg^{2+} and Ca^{2+} may not be explained in terms of the plasma-Ca^{2+}-, plasma-PTH-related hypothesis explicited for the homomorphism of the relationships between the post-placebo and post-clopamide 5 mg mean urinary flows of these cations. The reason for this impossibility resides in the lack of statistically significant effect of CLOP + PIND on urinary Ca^{2+} excretion during the first 3 hours after dosing with CLOP + PIND (Table I), that could have accounted for a change in plasma PTH concentration, and also in that the release of PTH

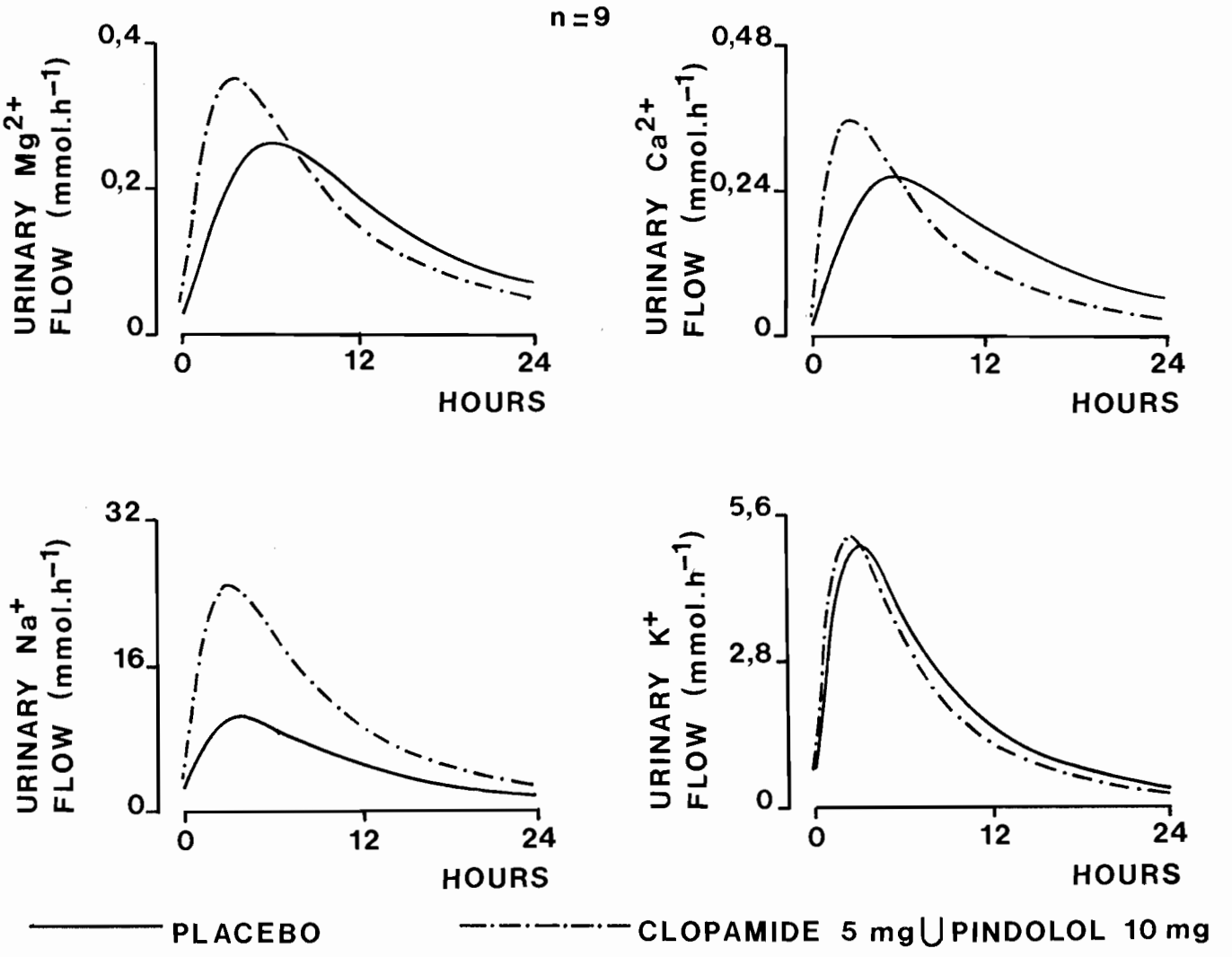

Fig. 5: Mean urinary Mg^{2+}, Ca^{2+}, Na^+ and K^+ flows after the separate administration of placebo and of a single dose of a combination of clopamide 5 mg and pindolol 10 mg (CLOP + PIND) per os to nine healthy volunteers at time 0 (0800 h). The relationships between the post-placebo and post-(CLOP + PIND) flows are homomorphic for Mg^{2+}, Ca^{2+} and K^+, i.e. each of these three relationships shows two analogous phases. The relationship between the post-placebo and post-(CLOP + PIND) flows of Na^+ exhibits an heteromorphic pattern with respect to the flow-relationship pattern common to Mg^{2+}, Ca^{2+} and K^+.

and its Ca^{2+}-mobilising action could have been diminished by pindolol, in so far as these events are known to be positively dependent on cAMP. This matter deserves further elucidation. The Na^+-flow relationship after placebo and CLOP + PIND was not different from that between placebo and clopamide 5 mg, perhaps because there is no mechanism whereby the addition of pindolol 10 mg could affect the prime natriuretic effect of clopamide 5 mg.

The relationships between the post-clopamide 5 mg and post-CLOP + PIND mean urinary flows of Mg^{2+}, Ca^{2+}, Na^+ and K^+ are compared in Fig. 6. Mean urinary Na^+ flows reveal that responses to the two formulations are similar in intensity and parallel in time, although that corresponding to clopamide 5 mg is consistently below the mean flow following CLOP + PIND. This difference, although statistically non significant, is directionally consistent and could be possibly due to a decrease in aldosterone-related Na^+ retention caused by pindolol. The limited degree of this difference confirms that the direct natriuretic effect of clopamide on Na^+ excretion outweighs the in-

Table III: Statistical features and parameter values of the linear transformations of the functions M(t). CLOP + PIND = combination of clopamide 5 mg and pindolol 10 mg.

Urinary variable	Unit used in calculations	Medication	Correlation of linearized M(t) r	Significance of r p	t_l (hour)	Original ordinate value of linearized M(t) a (hour)	Slope of linearized M(t) b	Time to peak excretion t_m (hour)
Chloride	mmol × 10	Placebo	0.9994	<0.0001	0.0765	0.7934	0.4022	3.67
		Clopamide 5 mg	0.9998	<0.0001	0.0420	0.5280	0.3632	2.93
		CLOP + PIND	>0.9999	<0.0001	0.0414	0.5342	0.3603	3.16
		Pindolol 10 mg	>0.9999	<0.0001	0.1024	0.8130	0.4066	3.70
Sodium	mmol × 10	Placebo	0.9996	<0.0001	0.1065	0.8985	0.4157	3.94
		Clopamide 5 mg	0.9999	<0.0001	0.0484	0.5415	0.3779	2.69
		CLOP + PIND	>0.9999	<0.0001	0.0475	0.5772	0.3716	3.17
		Pindolol 10 mg	0.9999	<0.0001	0.1543	1.0709	0.4093	4.69
Fluid	litre × 10^{-1}	Placebo	0.9998	<0.0001	0.0564	0.7024	0.3665	3.66
		Clopamide 5 mg	0.9999	<0.0001	0.0449	0.6185	0.3522	3.65
		CLOP + PIND	>0.9999	<0.0001	0.0347	0.5015	0.3555	2.92
		Pindolol 10 mg	>0.9999	<0.0001	0.0475	0.5698	0.3673	3.17
Potassium	mmol	Placebo	0.9994	<0.0001	0.0203	0.4881	0.3275	3.15
		Clopamide 5 mg	0.9996	<0.0001	0.0173	0.4935	0.3082	3.38
		CLOP + PIND	0.9998	<0.0001	0.0188	0.4151	0.3380	2.43
		Pindolol 10 mg	0.9999	<0.0001	0.0238	0.4413	0.3443	2.67
Calcium	mmol × 10^{-1}	Placebo	0.9965	0.0002	0.0484	0.9010	0.3303	5.80
		Clopamide 5 mg	0.9994	<0.0001	0.0295	0.5825	0.3391	3.63
		CLOP + PIND	0.9997	<0.0001	0.0290	0.4903	0.3539	2.67
		Pindolol 10 mg	0.9991	<0.0001	0.0509	0.8100	0.3301	4.85
Magnesium	mmol × 10^{-1}	Placebo	0.9961	0.0003	0.0507	0.9163	0.3275	5.81
		Clopamide 5 mg	0.9978	<0.0001	0.0280	0.6888	0.3185	4.59
		CLOP + PIND	0.9992	<0.0001	0.0311	0.5954	0.3394	3.63
		Pindolol 10 mg	0.9990	<0.0001	0.0508	0.7787	0.3418	4.59
Inorganic phosphate	mmol	Placebo	0.9943	0.0005	0.0527	1.0002	0.3353	6.29
		Clopamide 5 mg	0.9950	0.0004	0.0663	1.0838	0.3413	6.30
		CLOP + PIND	0.9989	<0.0001	0.0893	1.0984	0.3300	7.27
		Pindolol 10 mg	0.9964	0.0003	0.1200	1.3736	0.3293	8.96
Zinc	µmol	Placebo	0.9931	0.0007	0.0232	0.8033	0.2943	5.55
		Clopamide 5 mg	0.9984	<0.0001	0.1498	1.1258	0.4493	3.97
		CLOP + PIND	0.9975	0.0001	0.0149	0.5782	0.2948	4.10
		Pindolol 10 mg	0.9988	<0.0001	0.0227	0.5950	0.3181	3.87
Creatinine	µmol	Placebo	0.9968	0.0002	0.0780	0.9760	0.3854	4.87
		Clopamide 5 mg	0.9979	0.0001	0.0803	0.9401	0.3914	4.63
		CLOP + PIND	0.9982	<0.0001	0.0755	0.8880	0.3882	4.39
		Pindolol 10 mg	0.9984	<0.0001	0.0954	1.0077	0.3887	4.88
Urate	mmol × 10^{-1}	Placebo	0.9979	0.0001	0.0228	0.6319	0.3203	4.11
		Clopamide 5 mg	0.9990	<0.0001	0.0290	0.6318	0.3309	4.11
		CLOP + PIND	0.9993	<0.0001	0.0322	0.6353	0.3361	3.88
		Pindolol 10 mg	0.9992	<0.0001	0.0400	0.7337	0.3305	4.84

fluence of aldosterone. The relationships between the post-clopamide and post-CLOP + PIND Mg^{2+}, Ca^{2+} and K^+ mean urinary flows were homomorphic; a first phase in which the post-clopamide flow was below the CLOP + PIND flow was followed by a later phase favouring clopamide. As far as the urinary Mg^{2+} and K^+ flows were concerned, this overshoot could be explained by a decrease in plasma aldosterone provoked by pindolol when the combination was administered. A blockade of the adenylate cyclase system by pindolol at the parathyroid gland and renal levels would have entailed a decrease in the positive effect of PTH on Ca^{2+} reabsorption in the loop of Henle and a resulting increase in the excretion of Ca^{2+} after CLOP + PIND with respect to after clopamide 5 mg; since the case was directionally reverse, it is possible that the ISA of pindolol precluded the usual effects of beta-adrenergic blockade upon cAMP. Another explanation could be related to that although Ca^{2+} is mainly reabsorbed in the

Table IV: Serum variables before (hour 0) and after administration of single doses of placebo, clopamide 5 mg, a combination of clopamide 5 mg and pindolol 10 mg (CLOP + PIND), and pindolol 10 mg to nine healthy volunteers. Values as mean ± S.E.M.

Serum variable	Medication	Hours after dosing		
		0	6	24
Chloride ($mmol.L^{-1}$)	Placebo	107.0 ± 1.0	105.7 ± 1.4	106.6 ± 1.2
	Clopamide 5 mg	102.9 ± 1.3	102.6 ± 1.7	101.8 ± 1.7
	CLOP + PIND	105.2 ± 0.9	106.0 ± 0.6	102.4 ± 1.7
	Pindolol 10 mg	104.1 ± 0.5	105.3 ± 1.0	103.2 ± 1.1
Sodium ($mmol.L^{-1}$)	Placebo	140.7 ± 0.7	140.2 ± 0.5	140.8 ± 0.3
	Clopamide 5 mg	140.3 ± 0.9	139.0 ± 0.9	139.4 ± 0.9
	CLOP + PIND	141.0 ± 0.9	140.4 ± 0.9	141.0 ± 1.1
	Pindolol 10 mg	140.9 ± 0.8	141.7 ± 0.9	141.7 ± 0.9
Potassium ($mmol.L^{-1}$)	Placebo	4.08 ± 0.07	4.11 ± 0.11	4.41 ± 0.12*
	Clopamide 5 mg	4.14 ± 0.08	4.07 ± 0.12	3.90 ± 0.05*
	CLOP + PIND	4.24 ± 0.07	3.82 ± 0.07****	3.79 ± 0.09****
	Pindolol 10 mg	4.31 ± 0.10	4.11 ± 0.07	3.90 ± 0.08***
Calcium ($mmol.L^{-1}$)	Placebo	2.69 ± 0.05	2.63 ± 0.06	2.54 ± 0.06**
	Clopamide 5 mg	2.54 ± 0.10	2.63 ± 0.06	2.54 ± 0.09
	CLOP + PIND	2.63 ± 0.08	2.69 ± 0.08	2.70 ± 0.07
	Pindolol 10 mg	2.58 ± 0.08	2.54 ± 0.07	2.57 ± 0.06
Magnesium ($mmol.L^{-1}$)	Placebo	0.81 ± 0.01	0.82 ± 0.02	0.83 ± 0.02
	Clopamide 5 mg	0.85 ± 0.02	0.83 ± 0.02	0.83 ± 0.02
	CLOP + PIND	0.84 ± 0.02	0.83 ± 0.02	0.81 ± 0.02
	Pindolol 10 mg	0.88 ± 0.03	0.85 ± 0.02	0.85 ± 0.02
Inorganic phosphate ($mmol.L^{-1}$)	Placebo	1.43 ± 0.11	1.43 ± 0.05[a]**	1.36 ± 0.06
	Clopamide 5 mg	1.18 ± 0.11	1.28 ± 0.14	1.34 ± 0.09
	CLOP + PIND	1.31 ± 0.14	1.30 ± 0.10	1.34 ± 0.10
	Pindolol 10 mg	1.21 ± 0.12	1.37 ± 0.09	1.27 ± 0.08
Creatinine ($\mu mol.L^{-1}$)	Placebo	83.6 ± 10.9	97.2 ± 3.5	92.7 ± 3.5
	Clopamide 5 mg	102.7 ± 3.5	103.8 ± 4.0	106.2 ± 5.2
	CLOP + PIND	102.5 ± 2.5	104.3 ± 4.5	101.4 ± 2.5
	Pindolol 10 mg	98.5 ± 4.0	101.0 ± 4.6	99.6 ± 2.9
Blood urea nitrogen ($mmol.L^{-1}$)	Placebo	4.9 ± 0.2	4.4 ± 0.3*	5.7 ± 0.2**
	Clopamide 5 mg	5.0 ± 0.3	4.1 ± 0.2*	6.1 ± 0.3***
	CLOP + PIND	5.0 ± 0.3	3.9 ± 0.3***	5.5 ± 0.4
	Pindolol 10 mg	4.7 ± 0.3	3.8 ± 0.3**	4.8 ± 0.3
Urate ($mmol.L^{-1}$)	Placebo	0.36 ± 0.02	0.35 ± 0.02	0.37 ± 0.02
	Clopamide 5 mg	0.35 ± 0.02	0.36 ± 0.02	0.40 ± 0.02****
	CLOP + PIND	0.36 ± 0.02	0.37 ± 0.02**	0.40 ± 0.02****
	Pindolol 10 mg	0.35 ± 0.02	0.35 ± 0.02	0.38 ± 0.02****
Total CO_2 ($mmol.L^{-1}$)	Placebo	26.9 ± 1.0	26.2 ± 1.0	27.2 ± 1.0
	Clopamide 5 mg	25.6 ± 0.6	26.0 ± 0.7	27.1 ± 0.7**
	CLOP + PIND	24.3 ± 1.8	25.1 ± 0.8	25.8 ± 0.7
	Pindolol 10 mg	24.8 ± 1.0	24.7 ± 1.0	25.2 ± 0.3
Glucose ($mmol.L^{-1}$	Placebo	5.5 ± 0.3	5.6 ± 0.3	5.3 ± 0.1
	Clopamide 5 mg	5.2 ± 0.1	5.4 ± 0.4	5.4 ± 0.2
	CLOP + PIND	5.3 ± 0.1	6.1 ± 0.4	5.8 ± 0.1*
	Pindolol 10 mg	5.1 ± 0.2	6.4 ± 0.6	5.4 ± 0.1

[a]Data from four patients.
Significances of the differences with respect to hour-0 mean values: *$p < 0.05$; **$p < 0.02$; ***$p < 0.01$; ****$p < 0.001$.

loop of Henle it is also handled in the distal convoluted tubule, as proven by the fact amiloride has been found to promote its retention from preurine [33]. In consequence, the possibility that aldosterone plays a role in the renal regulation of Ca^{2+} deserves further basic research.

Clinical relevance of the findings

The potential risks of diuretic therapy secondary to Mg^{2+} and K^+ depletions include the development of cardiac arrhythmias, sudden death (mainly due to cardiac arrhythmias) and myocardial infarction [52, 54]. In addition, the deficiencies of these cations may interact with the effects of stress in a positive feedback manner [9, 15, 74], and perhaps also reduce the chronic effect of diuretics to some extent [13, 60], since Mg^{2+} exhibits vasodilatatory properties in man under certain circumstances [25] and a negative correlation between plasma Mg^{2+} and blood pressure has been found [48]. The importance of stress as a cardiovascular risk factor [63], in particular for sudden death in patients with ischaemic heart disease, is beyond debate [71]. Mental stress in hypertensives increases heart rate, augments cardiac output, causes vasoconstriction and diminishes the electrical stability of the heart. Magnesium deficiency also appears to be a mediator in the unfavourable alterations of carbohydrate metabolism and of the plasma lipid profile induced by diuretics [20, 49, 53].

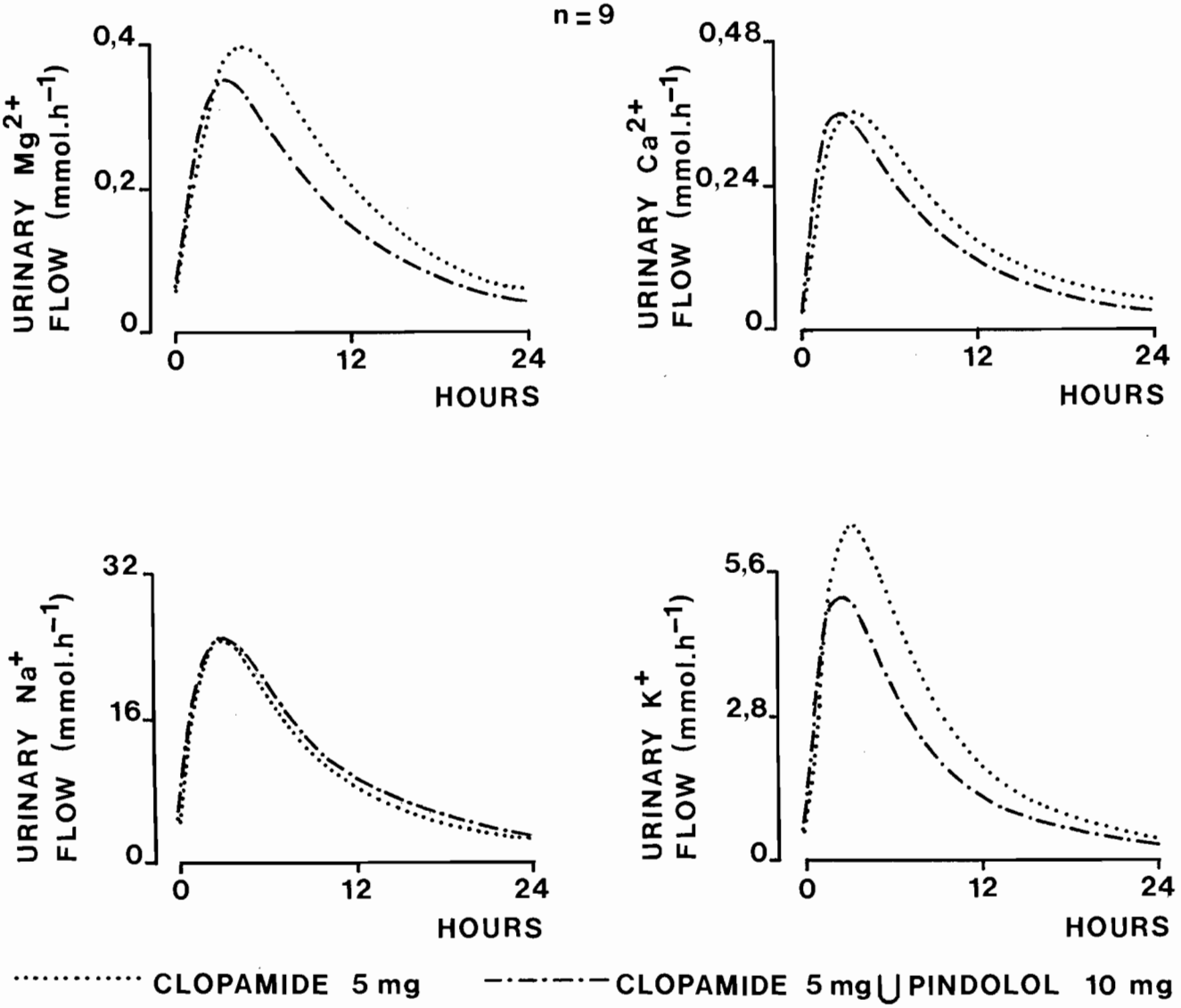

Fig. 6: Mean urinary Mg^{2+}, Ca^{2+}, Na^+ and K^+ flows after the separate administration of single doses of clopamide 5 mg and of a combination of clopamide 5 mg and pindolol 10 mg (CLOP + PIND) per os to nine healthy volunteers at time 0 (0800 h). The relationships between the post-clopamide and post-(CLOP + PIND) flows are homomorphic for Mg^{2+}, Ca^{2+} and K^+, i.e. each of these three relationships shows two analogous phases. The relationship between the post-placebo and post-(CLOP + PIND) flows of Na^+ exhibits an heteromorphic pattern with respect to the flow-relationship pattern common to Mg^{2+}, Ca^{2+} and K^+.

The counteraction of clopamide-induced hypermagnesiuresis and hyperkaliuresis by pindolol, when both drugs were administered in a fixed relationship of 5 and 10 mg respectively, constitutes the most favourable finding in a series of studies in which the effects of various diuretics, pindolol, captopril, and combinations of hydrochlorothiazide with acebutolol, amiloride, captopril, or sotalol were studied in accordance with a similar protocol. Table V shows the percentual changes with respect to control in mean urinary Na^+, K^+ and Mg^{2+} outputs recorded in the studies in question. If the percentual changes in mean 24-h urinary K^+ and Mg^{2+} outputs following each formulation are added, an arbitrary indicator of the potential risk entailed by therapy with each formulation is obtained (Table V). An increased risk is associated with the monocomponent diuretic formulations and the hydrochlorothiazide plus captopril and hydrochlorothiazide plus sotalol combinations studied. Low-risk formulations include captopril, pindolol, and three diuretic combinations, hydrochlorothiazide 12.5 mg and acebutolol 200 mg, hydrochlorothiazide 50 mg and amiloride 5 mg and clopamide 5 mg and pindolol 10 mg, the latter exhibiting the lowest risk except for pindolol alone.

The present findings, and those derived from similar 24-h studies in healthy individuals, do not prove that somatic balance of Mg^{2+} and K^+ will be necessarily unaffected by prolonged administration of formulations that do not exhibit magnesiuretic and kaliuretic actions in acute studies like the present. Disease-related, drug-related or other processes coincidental with therapy may give place to differences between the results obtained in acute experiments and those observed in long-term clinical application. The dose relationship between the components of a drug combination may not be ideal, with the result that hypermagnesiuresis occurs even in acute experiments similar to the present [35], or that additive effects of small, statistically non-significant, increases in daily Mg^{2+} or K^+ outputs become apparent following prolonged dosing with the formulation in question. An example of this is given by the recent finding that the acute administration of hydrochlorothiazide 50 mg and amiloride 5 mg caused a statistically non significant increase in mean 24-h urinary Mg^{2+} output [32], but it caused a statistically significant and clinically relevant fall of plasma Mg^{2+} in hypertensive patients after 20 weeks therapy [33]. The same combination has also been found to significantly decrease plasma K^+ concentration after a mean treatment period of 19.5 weeks [47].

Table V: Percentage changes in the mean 24-hour urinary outputs of Na^+, K^+ and Mg^{2+} with respect to control values, after the administration of single doses of various antihypertensive formulations to healthy volunteers.

Number of subjects	Drug and dose (mg)	$\%\Delta Na^+$	$\%\Delta K^+$	$\%\Delta Mg^{2+}$	$\%\Delta K^+ + \%\Delta Mg^{2+}$	Reference
13	Captopril 100 mg	− 3.3	− 4.8	17.6[b]	12.8	[38]
9	Chlorthalidone 100 mg	165.4[c]	69.6[b]	87.0[b]	156.6	[58]
9	Clopamide 5 mg	85.1[c]	25.4[a]	27.2[b]	52.6	[this publication]
13	Hydrochlorothiazide 25 mg	33.1[c]	20.5	73.4[c]	93.9	[38]
9	Hydrochlorothiazide 50 mg	63.0[b]	21.9[a]	43.8[a]	65.7	[31]
19	Hydrochlorothiazide 50 mg	61.0[c]	29.9[a]	24.5[a]	54.4	[32]
9	Pindolol 10 mg	− 3.9	−20.1	−14.5	− 34.6	[this publication]
13	Xipamide 5 mg	46.5[b]	40.6[b]	27.5[b]	68.1	[57]
13	Xipamide 10 mg	120.2[c]	55.4[b]	50.1[c]	105.5	[57]
13	Xipamide 20 mg	110.8[c]	79.5[c]	40.4[c]	119.9	[57]
9	Clopamide 5 mg and pindolol 10 mg	99.4[c]	−10.4	− 1.4	− 11.8	[this publication]
10	Hydrochlorothiazide 12.5 mg and acebutolol 200 mg	27.1[a]	12.2	4.3	16.5	[34]
13	Hydrochlorothiazide 50 mg and amiloride 5 mg	70.1[c]	− 5.8	8.2	2.4	[32]
13	Hydrochlorothiazide 25 mg and captopril 100 mg	74.8[c]	19.0[a]	45.1[c]	64.1	[38]
12	Hydrochlorothiazide 50 mg and sotalol 320 mg	65.4[c]	47.8[c]	19.6[a]	67.4	[35]

Significances of the differences between corresponding mean values after medication and during control: [a] $p<0.05$; [b] $p<0.01$; [c] $p<0.001$.

Various environmental and personal physiological and pathological factors which tend to decrease both somatic Mg^{2+} and K^+ may lead to deficiency of any of the cations per se, aggravate drug-induced losses or act together with drug therapy to produce the deficiencies. The occurrence of these factors should be controlled in any studies aimed at investigating the effects of chronic therapy with CLOP + PIND on Mg^{2+} and K^+ turnovers.

The increase in Zn^{2+} excretion induced by early distal tubular diuretics is liable to cause depletion of this cation, leading to relatively minor effects such as dysosmia and dysgeusia, to sexual impotence, which may be of significant degree, and to a retardation in wound-healing [61, 62]. If the latter applied to the healing of myocardial infarction, diuretic-induced Zn^{2+} depletion could become an important side effect of these drugs. Pindolol 10 mg counteracted clopamide-induced hyperzinciuresis (Table III), a fact which may prove of clinical relevance, although the paucity of information that exists on the renal handling of Zn^{2+} and on the effects of the cation on cardiovascular function precludes further speculation.

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