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ABSTRACT

Analgesics and nonsteroidal anti-inflammatory drugs (NSAIDs) are well recognized as a major class of therapeutic agent that causes renal papillary necrosis (RPN). Over the last decade a broad spectrum of other therapeutic agents and many chemicals have also been reported that have the potential to cause this lesion in animals and man. There is consensus that RPN is the primary lesion that can progress to cortical degeneration; and it is only at this stage that the lesion is easily diagnosed. In the absence of sensitive and selective noninvasive biomarkers of RPN there is still no clear indication of which compound, under what circumstances, has the greatest potential to cause this lesion in man. Attempts to mimic RPN in rodents using analgesics and NSAIDs have not provided robust models of the lesion. Thus, much of the research has concentrated on those compounds that cause an acute or subacute RPN as the basis by which to study the pathogenesis of the lesion. Based on the mechanistic understanding gleaned from these model compounds it has been possible to transpose an understanding of the underlying processes to the analgesics and NSAIDs. The mechanism of RPN is still controversial. There are data that support microvascular changes and local ischemic injury as the underlying cause. Alternatively, several model papillotoxins, some analgesics, and NSAIDs target selectively for the medullary interstitial cells, which is the earliest reported aberration, after which there are a series of degenerative processes affecting other renal cell types. Many papillotoxins have the potential to undergo prostaglandin hydroperoxidase-mediated metabolic activation, specifically in the renal medullary interstitial cells. These reactive intermediates, in the presence of large quantities of polyunsaturated lipid droplets, result in localized and selective injury of the medullary interstitial cells. These highly differentiated cells do not repair, and it is generally accepted that continuing insult to these cells will result in their progressive erosion. The loss of these cells is thought to be central to the degenerative cascade that affects the cortex. There is still a need to understand better the primary mechanism and the secondary consequences of RPN so that the risk of chemical agents in use and novel molecules can be fully assessed.

Keywords. Analgesic nephropathy; renal papillary necrosis; nonsteroidal anti-inflammatory drugs

INTRODUCTION

Renal papillary necrosis (RPN) was first described as a common condition in human diabetic patients, but there was a progressive realization that RPN has other causes (13), especially as a consequence of analgesic abuse, as many thousands of patients took excessive quantities of mixed analgesics in the 1960–1980s. Initially phenacetin was assumed to be the nephrotoxic common denominator (50, 51, 150, 151), and this compound was progressively banned from over-the-counter (OTC) products, but it is now appreciated that many analgesics and nonsteroidal anti-inflammatory drugs (NSAIDs) have papillotoxic potential if used in excessive quantities. It is also apparent that the problem of excessive use of analgesics continues today (13, 46, 56, 87, 96, 171, 185, 200, 201) and this healthcare cost is probably preventable (46, 47, 59). Analgesic abuse is a frequent cause of analgesic nephropathy that leads to end-stage renal disease and upper urothelial carcinoma throughout Europe (46, 47, 58–64, 179, 180, 203, 204), Australia (149–150, 171, 216), and other parts of the world (206–210). Interestingly, there are apparently only few cases in the USA (21, 29, 56, 96, 184, 200, 201, 226). The condition occurs in national or local clusters, for reasons that are not clear, but may relate to social customs, availability of specific medications, or other as yet poorly identified factors. Thus, national incidence for the condition tends to be low and does not adequately reflect the regional levels of renal disease that may occur. RPN in humans develops as a result of chronic consumption or long-term abuse of single and mixed analgesics. The lesion also occurs in adults and children who have been prescribed NSAIDs, and single analgesics, and it has also been implicated with a whole spectrum (Table I) of therapeutically used NSAIDs and some other drugs.

ANALGESIC NEPHROPATHY

The progression of renal failure in analgesic-associated nephropathy has been well documented (13, 32, 47, 78, 96, 142, 170, 171, 203, 204). Analgesic nephropathy is a degenerative condition leading to renal failure following long-term analgesic abuse. Pyelonephritis is a hallmark, as a result of an RPN. There are few clinical symptoms associated with the early development of analgesic-associated RPN (13, 32, 78, 171, 203, 204). The progression of renal damage is insidious and renal function may be severely compromised before the condition becomes obvious (13, 32, 78, 171, 203, 204).

1) Early symptoms include headaches, upper gastrointestinal disease such as peptic ulceration of stomach and duodenum, dyspepsia and anemia as a result of gastrointestinal tract bleeding, hemolysis, and iron deficiency. These are often seen in patients who have emotional dependence, anxiety, introversion, and neurosis.
2) Intermediate symptoms include urinary tract disease such as nocturia, dysuria, bacteriuria, sterile pyuria,

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TABLE I.—Chemicals and therapeutic agents reported to cause papillary necrosis in animals or linked to case reports of papillary necrosis in man.

Chemical or therapeutic agent	References
Acetaminophen	210
Antipyrine	111
Aspirin	30, 31, 39, 44, 113
Benoxaprofen	2
2,2-Bis(bromomethyl)-1,3-propanediol	65
2-Bromoethanamine	13
3-Bromopropanamine	183
Butylated hydroxyanisole	93
Cacodylic acid	165
2-Chloro-*N,N*-dimethylethanamine	183
2-Chloroethanamine	183
1-(2-Chloroethyl)-3-(trans-4-methylcyclohexyl)-1-nitrosourea	127
Cyclophosphamide	175
Dapsone	37
Dibromoneopentyl glycol	65
Diclofenac sodium	205
Dimethanesulfonate	176
Dimethylarsinic acid	165
Diphenylamine	135–137
Ethoxyquin	91, 146, 147
Ethyleneimine	13
Fenoprofen	109
Flurbiprofen	41, 167
Formaldehyde	222
Ibuprofen	212
Indometacin	2
Indomethacin	34, 154
Ipsapirone	188
Ketoprofen	2
Mefenamic acid	34, 36, 98, 209
Mesalazine	22, 169, 221
Mixed analgesics	47, 50–52, 61, 149–151, 170–172, 179, 180, 184
1-Naphthol	181
Naproxen	2, 35
Nefiracetam	117, 218
D-Ormaplatin	126
Pentazocine and aspirin	162
Phenacetin	30, 31, 71, 112, 140
Phenylbutazone	69, 88, 89, 116, 139, 177, 178, 186
N-Phenylanthranylic acid	92
o-Phenylphenol	211
Piroxicam	123, 124
Propyleneimine	76
1-(2-Pyrimidinyl)-piperazin	188
Rifampicin	130
Sulfuryl fluoride	55
Sulindac	2
Tepoxalin	125
Tolmetin	2
Triethanolamine	141

microscopic hematuria, ureteral colic, and lower back pains. It is now agreed that the primary lesion of analgesic abuse is in the medulla, where the "fine elements" such as the interstitial cells, endothelia, and loops of Henle are the earliest affected parts, leading to cortical degeneration and then leading to renal functional compromise and end-stage renal disease (13, 32, 78), changes that cannot currently be identified noninvasively. Some of the key pathological features include loss of the fine elements of the medulla (interstitial cells and microvasculature), changes in the medullary mucopolysaccharide staining, lipid accumulations as shown by Oil Red O, tubular dilatation and tubular casts, cortical scarring and glomerular sclerosis (13, 32, 78). Biochemical analysis shows a defect in ability to concentrate and acidify urine.

3) The late symptoms such as hypertension, cardiovascular disease, peripheral vascular disease, renal calculi and bladder stones and decreased glomerular filtration rate, increased blood urea nitrogen, renal tubular acidosis, and carcinoma of upper renal tract are common, but all these conditions could also have other causes. Even radiological examination may not identify papillary necrosis (if the necrosed papillae remains *in situ*), and even when loss of the papillae is obvious (in the presence or absence of other degenerative renal changes) the underlying cause still has to be established. Recently, the combined use of computerized tomography and ultrasound ensured a very high degree of positive diagnosis (28, 58, 63). The use of these diagnostic criteria has resulted in the increased incidence of previously undiagnosed RPN and analgesic nephropathy (64) in populations where pyelonephritis had previously been recognized but the underlying cause not identified.

Analgesic nephropathy is also reported in patients who have taken no phenacetin but who have abused acetylsalicylic acid, acetaminophen, and codeine (60, 174), and sales figures of analgesics are presented that suggest similarities between the occurrence of the disease and the sale of the most often consumed coformulated products (60, 174). These are thought to warrant legislative measurements to control analgesic consumption (62). These data are supported by the changing incidence of analgesic nephropathy in Australia where phenacetin was withdrawn and the potential to abuse coformulated mixed analgesics was limited (216). The data are controversial from a methodological point of view and there is an equally strong set of arguments that point to the role of the removal of phenacetin *per se* from the market as the single most significant force in reducing analgesic nephropathy (152, 153).

Epidemiological studies easily identify the adverse effects of long-term analgesic use or abuse (50, 51, 61, 179, 180, 200, 201). The diffuse symptoms, the protracted period of time over which the lesion develops, and the fact that it is difficult to gauge how much of which analgesic or NSAIDs was consumed over what period of time has precluded more detailed analysis of the epidemiological databases. While several reviews (20, 96, 97, 170–172, 182) have implicated acetaminophen and coformulated mixed analgesics (acetaminophen, aspirin, and caffeine), the epidemiological data that are available do not realistically allow such an interpretation (48).

Limitations on the Clinical Assessment of RPN

There are several reasons why progress in understanding the genesis of analgesic nephropathy and RPN has been so slow. A long incubation period, lack of biomarkers for an early diagnosis and the coformulation of multiple analgesics all tend to obscure the cause and effect. The role of any single anti-inflammatory drug in the development of RPN is difficult to confirm in man because of polypharmacy. This makes most epidemiological investigations too weak to be effective in identifying anything other than the abuse of phenacetin-containing

mixed analgesics that are a risk to humans. In addition, the changes in the formulation of mixed analgesics (marketed under the same name) has also served to confuse our understanding of what has been taken and the duration of abuse.

Analgesics and NSAIDs as the Causative Agents of RPN in Man

The compounds that cause RPN are still not fully identified. Several agents shown in Table I have been implicated in the lesion in humans. The effects of each compound in animals probably gives a better indication of the papillotoxic potential, but risk assessment is difficult.

The role of aspirin in RPN in humans is supported by a limited number of case reports where long-term ingestion of pentazocine and aspirin (162), aspirin abuse (163), and a case report of a child with rheumatic carditis who developed acute renal failure due to RPN after a short course of aspirin treatment (80). However, these effects are not universal and there was no evidence of significant renal dysfunction in patients with active lupus nephritis who took aspirin (mean total dosage 35 kg) continuously for 10 or more years (66). Rheumatology clinical studies indicate that the prevalence of nephropathy in habitual consumers of phenacetin-containing compounds is higher than that for habitual consumers of aspirin alone (29). One case control study demonstrates a low, but statistically significant risk of end-stage renal disease in association with aspirin, but all other case control studies and several prospective studies have been unable to identify a significant risk of chronic renal failure in patients using therapeutic doses of aspirin alone (44).

There are a number of clinical studies that identify RPN to be caused by the long-term use of NSAIDs as the sole or predominant medication (206), acetaminophen (210) and mixed analgesics (47, 50–52, 61, 149–150, 170–172, 179, 180), and both analgesic and NSAID use (185). By contrast, while phenacetin-containing mixed analgesics increase the relative risk of RPN, there is only a weak or no risk associated with acetaminophen *per se* (20, 149, 200, 201).

The Role of Caffeine in Mixed Analgesics

Recently, the controversy over the role of mixed analgesics has been fuelled by the publication of a position paper from the American National Kidney Foundation (95), in which the problems of the abuse of analgesics, the overuse of NSAIDs, and in particular the coformulation of mixed analgesics with caffeine were highlighted as a major cause of concern. This statement suggested that all medications containing 2 analgesics plus caffeine should be on prescription only.

This conclusion was reached on the basis of experimental and clinical publications that include the suggestions that caffeine is an "addictive or habituating" drug (59, 60, 62, 97, 174, 179, 180, 182), which is thought to explain the widespread use of caffeine-containing mixed analgesics. There is no consistent evidence to support such an assertion (108), which is particularly important as caffeine is so widely consumed in a whole range of beverages and foods. The epidemiological study of Pommer et al (179, 180) is also cited as showing increased relative risk of coformulated caffeine in mixed analgesics. This epidemiological evidence has been strongly refuted (48), and there are equally convincing arguments that phenacetin was key to the genesis of the lesion and its withdrawal has resulted in the decreasing healthcare importance of analgesic nephropathy (152, 153).

One of the strongest arguments to keep coformulated analgesics is that the addition of caffeine increases analgesic action 1.4-fold for a series of different types of mild to severe pain (1). Thus, it should be possible to reduce the intake of these medications and minimize the possible side effects in patients who adhere to the advised dose levels. In addition, there are drug safety data that do not identify any hazards from such coformulations (see below).

NSAIDs Linked to RPN in Man

There are case reports that link benoxaprofen (2), diclofenac sodium (205), fenoprofen (109), flurbiprofen (41, 167), ibuprofen (212), indometacin (2), indomethacin (154), ketoprofen (2), mefenamic acid (209), naproxen (2, 35), sulindac (2), and tolmetin (2) to the development of RPN in humans, and other NSAIDs have been implicated as causing the lesion in animals (Table I). There is still insufficient data from which to assess the hazard associated with these products, so that risk is uncertain but seems to be relatively small if used appropriately.

Other Therapeutic Agents Linked to RPN in Man

There are also case reports that link dapsone (37), mesalazine (221), pentazocine and aspirin (162), and rifampicin (130) to RPN in humans.

Animal Models of Papillary Necrosis

The difficulties in studying the genesis of RPN in humans has necessitated the increasing dependence on the use of animal models. The use of analgesics and NSAIDs has not, however, provided a useful tool for investigating the lesion as they suffer unidentified and uncontrolled factors that make biological variability large both within and between experiments. Risk assessment is made more difficult by the absence of plasma concentration data or quantitative and qualitative measurement of urinary parent drug and metabolites. Without such information the extrapolation of data to humans is made more difficult. Despite these difficulties, it has been possible to develop a better understanding of the molecular basis of papillary necrosis and the associated nephropathy using analgesics and NSAIDs. Much of the mechanistic research has been undertaken using model compounds that cause the lesion (see below) but have little or no structural relationship to therapeutic agents.

Medullary Structure and Function and Interspecies Extrapolation

The medulla is the least easily accessible part of the kidney and therefore poorly investigated in comparison to the rest of the kidney. The papilla is a small fraction of the renal mass that requires extensive care to assess

its pathology. It also provides little tissue for *in vitro* studies. This, in part, explains why many of the lesions that afflict this region of the kidney are still not adequately investigated or well understood. An in-depth review of the medulla's unique morphological (128, 131, 145, 148), functional (73, 145, 148, 161), and biochemical (25, 26, 45, 53, 54, 145, 213, 214) features is essential to understand how toxicants might affect it. The fine elements of the medulla include the loops of Henle, capillaries, interstitial cells, and the collecting duct epithelia embedded in a glycosaminoglycan-rich (13, 128, 131, 145, 148) matrix. Whereas the mouse, gerbil, rat, hamster, guinea pig, rabbit, dog, cat and primate kidney have only a single papilla, the pig and humans have up to 20 papillae in each kidney (13). The medulla concentrates the glomerular filtrate so that less than 1% leaves the kidney as urine (unless there is diuresis) by a series of processes that are still not completely understood, but there is considerable variability in the concentrating capacity of different species (13, 73).

Limitations on the Experimental Assessment of RPN

For the toxicologic pathologist, the problem of renal papillary necrosis extends well beyond the class of drugs with analgesic and NSAID activity. Many different molecules cause papillary necrosis during preclinical safety evaluation. Table I illustrates those molecules for which there are published data. The diversity of chemical structures (Fig. 1) makes nonsense of the idea that this lesion is limited to analgesic and NSAIDs only. Too often routine pathology assessment is not sufficiently exacting to section the papilla in such a way that focal lesions will be identified. Thus, it is likely that not all the chemicals with papillotoxic potential have been identified, and no observed adverse effects levels may be lower than currently indicated.

NSAID-Induced Papillary Necrosis in Animals

This may be due, in part, to gastric ulceration that is a frequent and often fatal early consequence of NSAID dosing (16).

Analgesics and NSAID Safety Screening in Animals

The administration of analgesics to animals has not produced robust, reproducible models of RPN, but all of the following compounds are sufficiently positive to warrant commentary.

Aspirin

Single, high intravenous (iv) or oral doses produces acute tubular necrosis of proximal tubules, rarely accompanied by RPN in susceptible strains. Chronic administration of aspirin alone for 68 wk in doses of 120–500 mg/kg/day caused RPN in rats (44). All male F344 rats fed aspirin for up to 68 wk in the presence or absence of *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide developed RPN and pelvic hyperplasia (39). This has been confirmed in male F344 rats treated with a combination of sodium saccharin and aspirin (113).

Aspirin (120–230 mg/kg/day) given continuously to female F344 rats for 40–83 wk caused ultrastructural changes in papillary interstitial cells and matrix in the midpapillary region, followed by changes to the thin limbs of the loop of Henle and medullary capillary endothelium (30, 31). After 18 wk recovery, there was no repair or proliferation of remaining undamaged type I medullary interstitial cells (30, 31).

Acetaminophen and Phenacetin

Whereas high doses of antipyrine (111) have been associated with RPN, it has been less commonly associated with acetaminophen or phenacetin (112). Generally acetaminophen has been regarded as a poor papillotoxin, whereas the superimposition of bacterial pyelonephritis was needed to produce the lesion (71). No hamsters given acetaminophen (up to 400 mg/kg) developed RPN (34). Recently, however, it has become apparent that long-term acetaminophen (140–210 mg/kg/day) administration to female F344 rats for 40–83 wk caused RPN, which is associated with irreversible damage to the medullary interstitial cells and the rest of the interstitium (30, 31).

Mixed Analgesics and Their Coformulation with Caffeine

There are many studies in the literature where mixed analgesics have been administered, but there is no consensus over the papillotoxic potential of multiple analgesics in the presence or absence of caffeine. Some reports suggest that the coformulation of acetaminophen and aspirin cause RPN, where each on their own did not (52, 170–172, 184). There is also evidence that the inclusion of caffeine reduced the papillotoxicity of phenacetin (140), but there was no indication of enhanced toxicity between phenacetin and aspirin and each or both of these and caffeine. More recently, the administration of aspirin, acetaminophen and caffeine individually or in different combinations has shown no toxicological interactions between the analgesics or between them and caffeine (133). The pharmacokinetic profiling of aspirin, acetaminophen, and caffeine demonstrated that there were no interactions between the products, and plasma levels were consistent with normal therapeutic use in humans. The coadministration of aspirin and acetaminophen apparently caused no greater RPN than either compound on its own (30, 31). Taken together, these data do not support any additive or synergistic interactions between aspirin, acetaminophen (or phenacetin), and caffeine in any of the combinations investigated.

There is little evidence for interactions between acetylsalicylic acid, acetaminophen, and caffeine that could be of toxicological significance. The reduction in mouse kidney glutathione (GSH) following aspirin and acetaminophen (150–600 mg/kg) showed no interactions when both analgesics were administered, and the inclusion of caffeine had no effect (68). Similarly, the well-established effect of acetylsalicylic acid in reducing renal prostaglandin E_2, 6-keto-prostaglandin $F_{1\alpha}$, was less marked for paracetamol, but the combination of the analgesics with or without caffeine had no effects (67).

NSAIDs

Renal toxicity of NSAIDs has been reviewed (23, 24, 56, 227). Despite the well-recognized papillotoxic poten-

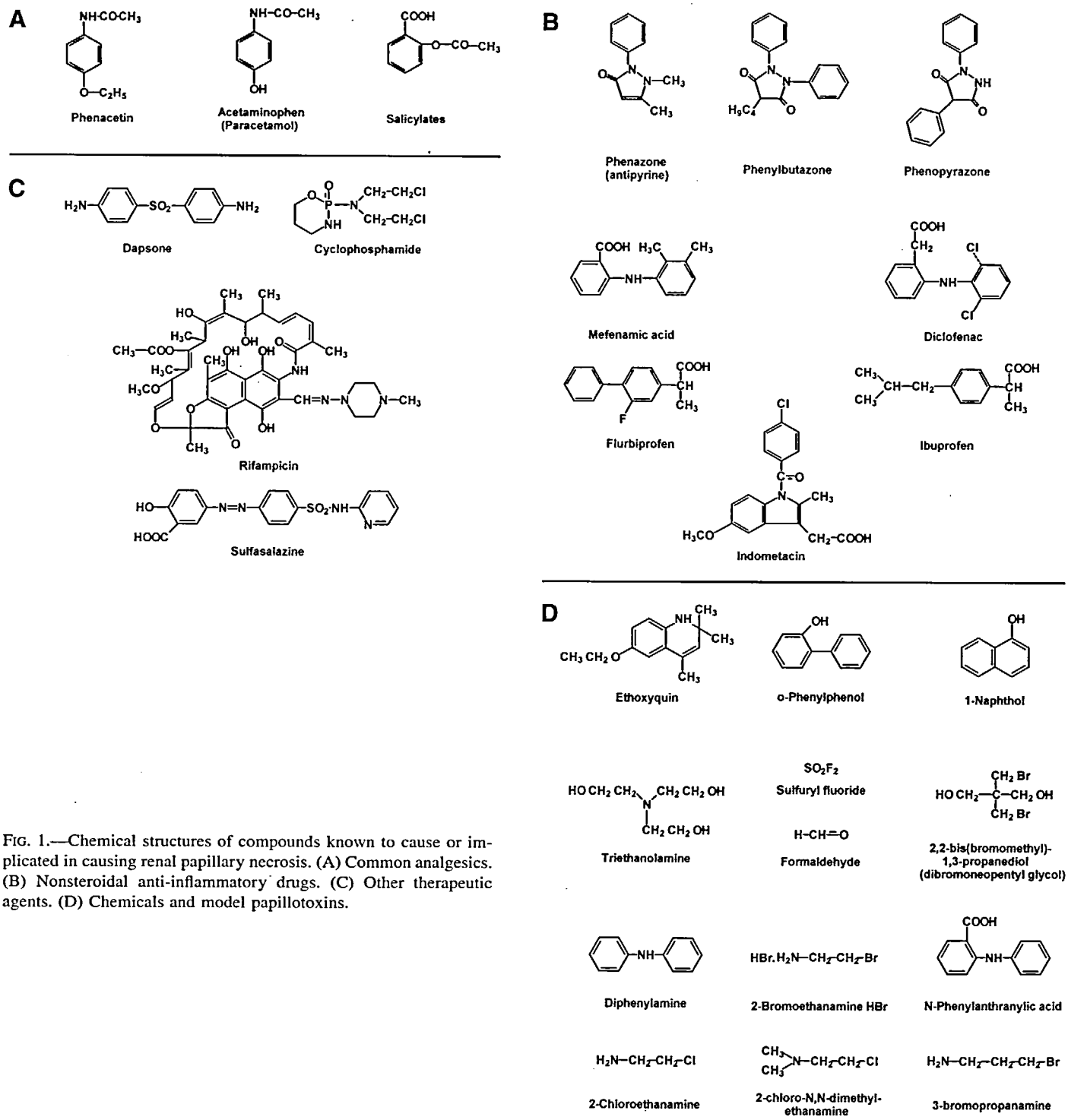

FIG. 1.—Chemical structures of compounds known to cause or implicated in causing renal papillary necrosis. (A) Common analgesics. (B) Nonsteroidal anti-inflammatory drugs. (C) Other therapeutic agents. (D) Chemicals and model papillotoxins.

tial of these compounds, there are few data by which to compare them, and there is a paucity of data on the progression of the lesion they cause, as most often changes are assessed at the termination of long-term studies where there have been few if any interim investigations.

Phenylbutazone

The papillotoxic effects of phenylbutazone are well described in horses (69, 139, 186), especially when water deprived (88, 89), but there are virtually no data on the pathogenesis or mechanism of its action. Both F344/N rats and B6C3F1 mice given phenylbutazone (50 or 100 mg/kg for rats and 50–300 mg/kg) 5 days per week over 2 yr showed a dose-related RPN. The frequency of RPN, pelvic dilatation and mineralization, cysts, and pelvis epithelial hyperplasia were greater in the female F344/N rats (116). While there are marked and very significant interspecies differences in nephrotoxicity following 200

mg/kg phenylbutazone once daily to Wistar, Lister-Hooded, Sprague-Dawley, and F344 rats, the effects on the papilla are more subtle (177). Wistar and Lister-Hooded rats seem to tolerate phenylbutazone better than Sprague-Dawley and F344. There also seem to be some sex-related differences as Sprague-Dawley males are more sensitive than females. Where RPN occurred, the lesion was indistinguishable between the species (177, 178).

Other Therapeutic Agents

Piroxicam administered at 1 and 1.5 mg/kg *per os* (po) every 48 hr for 30 and 14 days, respectively, followed by 0.5 mg/kg every 48 hr for up to 6 mo (123) and doses as low as 0.3 mg/kg po every 48 hr for several months caused RPN (detected at necropsy) in dogs (124). Mefenamic acid is a most potent papillotoxin in rats (36, 98) and the Syrian hamster (34) where indomethacin also caused RPN (34). Mesalazine causes RPN in several species (22, 169).

NSAIDs and Caffeine

There are reports (36, 98) that the coformulation of mefenamic acid and caffeine exacerbate RPN, but mefenamic acid is the most papillotoxic of the NSAIDs (34) and the observation has apparently not been repeated.

Other Compounds Causing RPN

In the last decade, there have been numerous compounds identified to cause RPN. Most often, there is a single mention of these pathology findings in a long-term study. Rarely is there any mechanistic or time-course information. Some of the data represent one or a few animals with RPN at the highest dose group, and it is these compounds that are likely to be of little mechanistic interest. There are, however, other compounds where their potential to undergo metabolic activation or nature of development of the lesion (dose-related or rapidly developing) does offer the potential for additional insight into the processes that underlie the lesion.

Anticancer Drugs

A single 250-mg/kg subcutaneous (sc) injection of 1-(2-chloroethyl)-3-(trans-4-methylcyclohexyl)-1-nitrosourea to male F344 rats caused a massive RPN within 7 days, with only limited necrosis to the proximal tubules, whereas 40 mg/kg chlorozotocin (2-[3-(2-chloroethyl)-3-nitrosoureido]-D-glucopyranose) initially resulted in acute tubular necrosis of the proximal tubules of the cortex, followed later by necrosis of papillary collecting ducts (127). Cyclophosphamide caused RPN (38–83% incidence), pyelonephritis, hydroureter and hydronephrosis (175).

Novel Therapeutic Agents

High doses (1,250 mg/kg/day) of 6-amidino-2-naphthyl-4-[(4, 5-dihydro-1H-imidazol-2-yl)-amino]benzoate dimethanesulfonate, an imidazole protease inhibitor, given to Sprague-Dawley rats po for 13 wk caused RPN, but there were no nephrotoxic effects using doses ≤250 mg/kg/day (176). Tepoxalin (5-[4-chlorophenyl]-*N*-hydroxy-1-[4-methoxyphenyl]-*N*-methyl-1H-pyrazole-3-propanamide) an orally active NSAID (that inhibits both cyclooxygenase and 5-lipoxygenase activities) caused RPN in a dog given 15 mg/kg/day for 6 mo. Changes were less well defined in rats (125). High doses (180 mg/kg) of nefiracetam (*N*-[2, 6-dimethylphenyl]-2-[2-oxo-1-pyrrolidinyl] acetamide), a cognition-enhancing agent, given to Beagle dogs of both sexes for up to 13 wk po caused RPN and epithelial degeneration and hyperplasia in the papilla and collecting duct and interstitial congestion, which were reflected in the clinical abnormalities of hematuria and increased protein excretion in urine. There were no signs of nephrotoxicity using 60 mg/kg of nefiracetam (117, 218). The serotonin antogonist ipsapirone given at doses of 4,500 ppm to Wistar rats and its metabolite 1-(2-pyrimidinyl)-piperazin cause a marked cellular swelling and vacuolation of the medullary and papillary collecting duct within 1 wk of treatment, focal RPN by 13 wk, and complete necrosis of the papilla following longer treatment (188). RPN was attributed to collecting duct changes as a result of local action of increased endothelin on the collecting ducts.

Antioxidants

Male F344 rats maintained on 2% butylated hydroxyanisole for 52 wk showed RPN (93). Ethoxyquin (0.5% in diet) accumulates in the kidney (121) and is nephrotoxic in rats (146, 147). Hard and Neal (91) studied the sequence of renal change in young F344 rats fed 0.5% ethoxyquin-containing diet for up to 18 mo. The primary lesion was RPN in the male rat, commencing as interstitial degeneration of the papillary tip by 4 wk and progressing to total RPN by 24 wk, including cortical degeneration and urothelial hyperplasia in the renal pelvis. Female rats developed papillary change at a later stage than males (91, 147), which never progressed beyond interstitial degeneration (91). The biological effects of ethoxyquin include inhibition of mitochondrial electron transport (187) and combined cyclooxygenase/lipoxygenase inhibition (155, 215). The compound undergoes oxidative metabolism and is excreted as the parent compound and both hydroxylated and dihydroxylated ethoxyquin in rat urine (122).

Other Chemicals

A 2-yr drinking-water study of formaldehyde given to male and female Wistar rats (1.2, 15, or 82 mg/kg and 1.8, 21, and 109 mg/kg/day, respectively) showed RPN in both sexes at 105 wk. The lesion, not reported at 53 or 79 wk, was attributed to prolonged lowered water intake, which was about 50% of the control consumption (222), representing an attractive but simplistic explanation. Both *o*-phenylphenol and Na-*o*-phenylphenate (but not diphenyl) given po as a 2% diet to male F344 rats caused RPN within 4 wk, which progressed to include regenerating hyperplasia by wk 16 and 24. Na-*o*-phenylphenate also caused a pronounced pelvic urothelial hyperplasia (211). Charles River CD1 mice given a single dose of 1-naphthol (1 g/kg) po developed degeneration of the distal tubule epithelium, tubular dilatation, and RPN 2 wk after dosing, which was attributed to intravascular thrombosis. By contrast, repeated dosing for 30

days (50–200 mg/kg) caused no nephrotoxicity (181). Exposure to 600 ppm sulfuryl fluoride (a structural fumigant) for 6 hr/day, 5 days/wk caused the death of numbers of male F344 rats and severe RPN and epithelial hyperplasia. In female rats that survived 2 wk exposure, only epithelial hyperplasia was present. There were no renal changes in male or female rats exposed to 300 ppm or New Zealand White rabbits exposed to 30–300 ppm sulfuryl fluoride using the same exposure protocol for 13 wk (55). Triethanolamine (1 and 2% in distilled water *ad libitum* for 69 wk, then half this concentration, due to nephrotoxicity, for the rest of a 2-yr study) caused a dose-related nephrotoxicity to male and female F344 rats. This was most notable in females where chronic nephropathy, mineralized papillae, pyelonephritis, RPN, and hydronephrosis were all marked, whereas males showed chronic nephropathy and papillary mineralization but few other changes (141). The flame retardant 2,2-bis(bromomethyl)-1,3-propanediol (dibromoneopentyl glycol) at 800 mg/kg po or 5,000–20,000 ppm in the diet for 13 wk caused RPN in both male F344/N rats and B6C3F1 mice, but lower doses had no effects. Mice were more sensitive than rats, and male rats and mice were more sensitive than females (65).

Organometallic Compounds

Two organometallic compounds cause RPN. The pure isomer D-ormaplatin (of the racemeric tetraplatin) given iv at 4 and 9 mg/kg caused a dose-related, acute RPN and pelvic urothelial hyperplasia (and also cortical injury) similarly in both male and female F344 rats (126). Dimethylarsinic acid (cacodylic acid, given po at doses of 113, 85, and 57 mg/kg to F344/DuCrj rats) caused RPN and papillary urothelial hyperplasia by 4 wk and also proximal tubular degeneration and necrosis. Mortality was higher and appeared more quickly in females than in males, and while the cortical lesion was similar in both sexes. The RPN and hyperplasia were more frequent in males, possibly due to their longer survival (165).

Multichemical Interactions

There are also examples where the coadministration of 2 substances affected the expression of papillotoxic potential. Male B6C3F1 mice given a single dose (80 mg/kg) of *N*-nitrosodiethylamine intraperitoneally (ip) were exposed to a diet containing di(2-ethylhexyl)-phthalate (6,000 ppm), butylated hydroxyanisole (7,500 ppm), or indomethacin (10 ppm in the drinking water) alone or in combination for 29 wk. Mice exposed to indomethacin-phthalate showed a high incidence of RPN that was not found in animals treated with indomethacin alone or di(2-ethylhexyl)-phthalate alone (90). F344 male rats treated with a combination of sodium saccharin and aspirin had a higher incidence of RPN than rats treated with aspirin only (113). Male F344 rats maintained on a diet containing 2% butylated hydroxyanisole and 0.25% retinyl acetate in drinking water for 52 wk showed severe RPN and nephrocalcinosis. In addition, 2% butylated hydroxyanisole on its own caused RPN. There were no data on retinyl acetate on its own (93).

TABLE II.—Animal species and strains in which experimental renal papillary necrosis has been reported.

Rats:	Wistar, Fischer 344, Sprague-Dawley, Lister-Hooded, Holtzman, Gunn, heterogeneous Battleboro, *Mastomys*, Munich Wistar Froemter rats
Mice:	Balb/c, B6C3F1, CD-1, C57Bl/6 mice, MF1-nu/nu/Ola/Hsd nude, Obese, Schnider, Swiss ICR
Other rodents:	Mongolian gerbil, Syrian hamster
Other laboratory animals:	Beagles
Primates:	Marmoset, baboon
Domestic animals:	Large White pig, horses

MODEL PAPILLOTOXINS

There are molecules that offer the experimental pathologist the opportunity to induce RPN in all treated animals within a short period. The greatest success in the study of RPN has been gained from the use of a limited number of compounds that produce a model lesion in laboratory animals (Table II). Administration of these compounds to rats satisfies the requirements of experimental pathology (17) and causes RPN and other physiological and pathological changes such as altered medullary mucopolysaccharide matrix staining (15, 16, 82, 85, 159) and Oil Red O-positive lipid material, which is especially marked in epithelial cells (16, 18, 83, 160). These changes are also seen in the kidneys of human analgesic abusers (13, 32, 78, 164). These models have helped study of the progression of papillary necrosis from the first cell-specific injury in the medulla, through a series of secondary changes causing marked damage in the cortex.

Diphenylamine

There have been interesting advantages in the understanding of the molecular basis of the diphenylamine-induced lesion (135–137). The early ultrastructural lesions have been described in Syrian hamsters given 600 mg diphenylamine/kg po as a single dose. Initial lesions were observed in the endothelial cells of the ascending vasa recta in the proximal portion of the renal papilla 1 hr after diphenylamine administration. The endothelial cell basal plasma membrane was elevated from the basal lamina, forming large subendothelial vacuoles. Alterations in inner medullary interstitial cells, endothelial cells of the descending vasa recta, and the epithelial cells of the thin limbs of Henle and the medullary collecting tubules were observed subsequent to the ascending vasa recta lesion. It was concluded that the endothelial cell of the ascending vasa recta is the target cell in diphenylamine-induced renal papillary necrosis in Syrian hamsters (137).

N-Phenylanthranylic Acid

The interesting feature of *N*-phenylanthranylic acid is its close structural analogy with the fenamic acid

NSAIDs, but having the advantage of lower propensity to cause gastric ulceration. Oral gavage causes a dose-related RPN in 7–14 days (92), and a single ip injection of the sodium salt causes an acute RPN within 24–48 hr (P. H. Bach, unpublished data).

Haloalkylamines and Imines

Ethyleneimine was the first of the prototype compounds studied, but its explosive instability and powerful mutagenic and alkylating activity (49) has stopped its use [see Bach and Bridges (13) for review]. Recently, the use of propyleneimine has been reported (76). 2-Bromoethanamine has been used so widely that it is considered as a separate topic.

2-BROMOETHANAMINE: THE MOST WIDELY USED MODEL FOR INDUCING RPN

2-Bromoethanamine (BEA) hydrobromide (also called 2-bromoethylamine) has the advantage of being chemically stable and has been used as the most popular model papillotoxin. Although it is structurally unrelated to the analgesics or NSAIDs, its distinct advantage is that a single injection (iv, ip or sc) causes a dose-related RPN within 24–48 hr (13). Its analogues, 3-bromopropanamine, 2-chloroethanamine, and 2-chloro-*N,N*-dimethylethanamine cause RPN with decreasing potency (183).

Functional Changes Caused by BEA

The renal functional changes following BEA closely parallel those reported for human analgesic abusers (13, 16, 17) and include loss of urinary concentrating ability (15, 192–199, 225), electrolyte wasting (192–199, 225), and severe cortical degeneration, which is a late but consistent secondary consequence of the medullary lesion (13, 192–199, 225).

BEA as a Tool for Renal Physiology

BEA has been so successful in ablating the papilla in rodents that it has become a widely used tool by renal physiologists. Specifically, many of the early changes appear to have their genesis within the first few hours after dosing.The effects of BEA on cellular and renal function have been reviewed (7, 192, 193), and specifically this compound has been used to study the effects of RPN on, or the role of the renal papillae in, natriuresis (110); carbonic anhydrase independent bicarbonate reabsorption (42); regulation of sodium excretion (38), urea and Na^+ excretion, osmolality, glomerular filtration rate and *p*-aminohippurate clearance (229), atrial natriuretic factor (223), the natriuretic effect of atriopeptin III (74), natriuretic response to hypervolemia (118), blood pressure in normotension and spontaneous hypertension (94), systemic acid–base balance (168), and atrial natriuretic factor (99). BEA has also been used to study the hemodynamic consequences of RPN (190), renal clearance of cephalexin in renal failure (144), prediction of the renal clearance of cimetidine using endogenous *N*-1-methylnicotinamide (143), and the role of a single enzyme of the *de novo* pathway for the biosynthesis of platelet-activating factor in the medulla (132, 225)

The Pathologic Course of BEA-Induced RPN

The pathophysiological course of BEA-induced RPN closely parallels the early and subsequent morphological and functional changes reported in animals dosed with analgesics and NSAIDs and human analgesic abusers (13).

The first cell types to undergo degenerative changes are the medullary interstitial cells at the tip of the papilla. Subsequently, more of the interstitial cells are affected toward the corticomedullary junction, and later (12 hr) the collecting duct epithelia and other areas of the distal nephron show degenerative changes, and more widespread necrosis becomes apparent. The cortex is also affected, characterized by hydropic changes in proximal tubules (15) and the progressive loss of alkaline phosphatase, γ-glutamyl transpeptidase, and adenosine triphosphatase from the brush border (85). Papillary necrosis is associated with the progressive deposition of neutral lipid material in the capillaries, collecting duct, and covering epithelial cells. Lipid staining extends into the outer medulla that would appear as normal by routine staining (15, 16, 19). The lipid staining of epithelial and microvascular cells for neutral lipid appears to be pathognomonic for RPN, as it also occurs in the pig (83), baboon, and marmoset following BEA (Bach and Gregg, unpublished observations) and aspirin-induced (160) RPN. By contrast, chemicals that affect the cortex (e.g., hexachlorobutadiene and cis-platin) do not produce specific localized lipid changes in the medullary epithelial cells (19).

It has been assumed that microvascular changes play a role in medullary anoxia and necrosis (43, 230, 231), but this does not appear to be the case in low-dose BEA-induced RPN in Wistar rats. Platelet adhesion is not seen before 8 hr after a single dose of BEA. Platelets then increase markedly, but only in those capillaries adjacent to necrosed areas (82). Colloidal carbon outlines microvascular filling and shows how this changes as a result of renal injury. After BEA there is a shift of cortical microvascular filling to the outer medulla (up to 4 hr) but a shift of colloidal carbon to the inner medulla from 8 to 26 hr. The necrosed medulla is avascular 48 hr after BEA, but capillaries are always patent beyond the regions in which RPN had occurred, confirming the absence of microvascular occlusion (15). Monastral Blue B demonstrates a fully maintained capillary integrity and the absence of plasma leakage into the interstitium (16, 82).

Metabolism of BEA

There are limited data on the metabolism of radiolabeled BEA (11). Following ip administration, BEA is absorbed into the stomach and label distributed to the bladder, stomach, upper gastrointestinal tract, and kidney, with significant amounts also present in the liver (14). Murray et al (166) suggested the cyclization of BEA to ethyleneimine as a mandatory step in the process of RPN, but chromatographic data (232) exclude this, as does the absence of a volatile BEA-derived radiolabeled component in urine and breath of rats (P. H. Bach, unpublished data). This suggests that no ethyleneimine is excreted *per se*. Recently, ^1H NMR spectroscopy has been used to

demonstrate that haloalkylamines react with bicarbonate ions to form *N*-carbamates and 2-oxazolidones (4). BEA also cyclizes to an alkylating aziridine in cell culture media and blood plasma (4, 105). Urine contains BEA itself and 2 novel metabolites 2-oxazolidone and 5-hydroxy-2-oxazolidone, probably formed by reaction with endogenous bicarbonate followed by a cyclization reaction eliminating HBr (4, 105).

Species, Strain, and Sex Differences

BEA produces similar papillary lesions in many different species and several strains of rodents (Table II). It is difficult to identify sensitive and resistance species or strains as few investigations compared different animals at the same time. There are convincing data to show that F344 rats are more sensitive to BEA (150 mg/kg) than *Mastomys* (102), but the same is true for mercuric chloride (103). C57Bl/6 mice are more sensitive than Obese mice, which are in turn more sensitive that Balb/C mice. This difference in sensitivity could represent the relative levels of BEA (given at 100 mg/kg ip) reaching the kidneys based on the kidney : body weight ratios (202). Male MF1-nu/nu/Ola/Hsd nude mice (81) and Swiss ICR mouse (231) have an atypical response to BEA that includes both RPN and total necrosis of the S2 and S3 segments. There were no sex-related differences in BEA-induced RPN in the Mongolian gerbil (230).

The Diagnosis of Renal Papillary Necrosis

Attempts to diagnose RPN noninvasively have been based on several different approaches, but to date there is still a requirement for painstaking histopathology, preparation of the kidneys, and careful assessment of sections, especially if focal lesions are present.

Conventional Urinalysis

Stonard et al (217) reported that *N*-acetyl-β-glucosaminidase (NAG) showed a sustained elevation after papillary damage, but this is not pathognomonic for the site of injury and there was no relationship between the severity of the lesion and the elevation of NAG. BEA causes diuresis, failure to concentrate urine and electrolyte imbalances, but these are common with many different types of renal injuries. Thus there were no commonly used criteria of urinalysis that identified RPN *per se,* although some of these parameters showed that changes were transiently associated with this lesion.

Nuclear Magnetic Resonance Imaging

Early work with nuclear magnetic resonance imaging (NMRI) showed that there were marked differences between the characteristics of the medulla and cortex, and BEA caused a significant increase in kidney size (as shown by T_2 images) and also an increase in the medulla to cortex T_1 ratio (33, 70). The resolution in these systems was, however, poor, and the advent of magnetic resonance microscopy (95) provided resolution high enough to demonstrate that T_2 was elevated (as a reflection of the water content) in the inner stripe of the outer medulla 48 hr after BEA (150 mg/kg). There were, however, no changes in the inner medulla. Magnetic resonance microscopy (114) will continue to advance as a practical tool that should be able to identify such lesions in the future and follow their subsequent changes and how this affects the kidney.

NMR Spectroscopy

High-resolution ^{1}H NMR spectroscopy of urine and other biological fluids offers the potential to assess metabolic changes as they occur. BEA-induced papillary insult resulted in elevations in urinary trimethylamine *N*-oxide, dimethylamine, acetate, and succinate (76). Recently, a transient elevation in urinary glutaric acid and adipic acid (105) has been reported and abnormal urinary metabolite profiles similar to humans with glutaric aciduria type II. This is caused by a lack of mitochondrial fatty acyl coenzyme A dehydrogenases, suggesting that BEA may target this enzyme. It has also been possible to follow the sequential changes following renal insult (101) and assess urinary concentrations of 20 endogenous substances simultaneously by NMR at different time points, to provide a time-related multidimensional description of the urinary biochemistry for each rat (104), but the data are complex and difficult to interpret. The development of automatic data reduction and pattern recognition methods for analysis of ^{1}H NMR spectra (4, 77, 78, 104, 106, 107) have clear potential to assist in diagnosis, which could be improved by artificial neural networks (5). The technique is, however, relatively insensitive in terms of the high concentrations of urinary material that are measured, and the fact that only gross lesions have so far been studied. This analytical approach does not appear to have been applied to humans with renal lesions.

Kidney-Derived Antigens

There are changes in urinary enzymes and kidney-derived antigens after acute RPN in rats (27), but it is not clear if these antigens are either sufficiently sensitive or selective, and also their appearance relates only to the acute phase of the lesion.

Lipid Histochemistry and Urinary Lipid Analysis

Based on the finding of changes in medullary and cortical lipid histochemistry (32, 78, 159, 160), investigation of urine from BEA-treated animals shows an increase in triacylglycerols (19), and more recently an increase in total phospholipids caused by BEA and *N*-phenylanthranylic acid (Fig. 2).

The Mechanism of BEA Target Selective Toxicity

The molecular basis of BEA target selectivity remains controversial, and opinion is divided between vascular injury and direct and selective medullary interstitial cell cytotoxicity.

Vascular Effects

Early studies [see Bach and Bridges (13) and Bach and Gregg (16) for review] highlighted microvascular changes in chronically induced RPN. Under these circumstances, however, it was doubtful if it was possible to differentiate microvascular changes as "cause" or

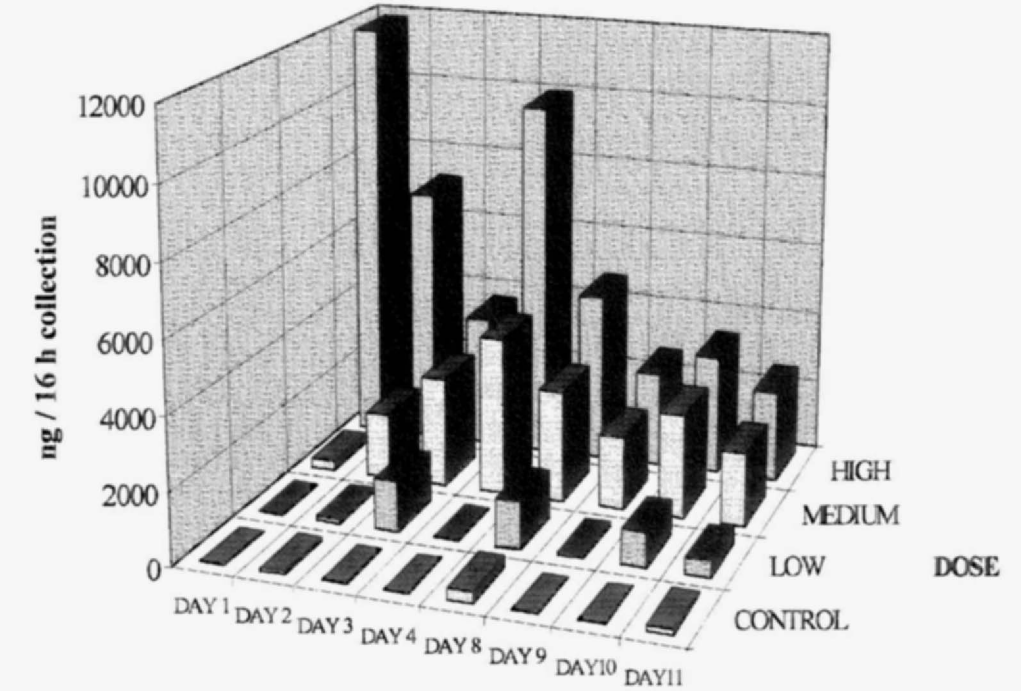

FIG. 2.—Changes in total urinary phospholipids following oral dosing with *N*-phenylanthranylic acid at levels of 100, 250, and 500 mg/kg po on days 1–4 and 8–11, with a respite on days 6–10, compared to control animals. Urinary samples (collected for 16 hr per day) were extracted with chloroform:methanol and lipid profiles assessed in 20 µl of extract, delivered by a Camag Linomat applicator to Merck silica gel high performance thin layer (HPTLC) plates. The plates were developed with standard reagents in a Camag horizontal developing chamber, and separated bands were stained using the manganese chloride-sulfuric acid reagent heated in an oven for 40 min. The colored bands were quantified using a Desaga CD-60 densitometer scanned at optimized conditions using lipid standards (Sigma, Poole).

"consequence" of RPN. The acute nature of the BEA-induced lesion does, however, offer the potential to define the relationship between microvascular injury and RPN. Microangiographic studies in the rat (43) revealed reduced vasa recta perfusion, and tubular injection studies showed unobstructed tubules and collecting ducts, supporting vasoconstriction. This study did, however, use high doses of BEA and only assessed the microvasculature at an advanced stage of renal injury. Light microscopic and ultrastructural changes in Mongolian gerbils given 250 mg/kg BEA (230) supported alterations restricted to the vasa recta and occlusion by platelets adherent, suggesting ischemic necrosis of the inner medulla that develops secondary to endothelial damage. Similarly, 300 mg/kg BEA caused an ischemic necrosis that was initiated by endothelial cell damage in the Swiss ICR mouse (231). These data supporting microvascular changes as the initiating factor in RPN may not be representative because of the high concentrating capacity of Swiss ICR and Mongolian gerbils and the atypical response of the ICR mouse (where there is also proximal tubule necrosis). Each of these studies has used high doses of BEA, the nonpapillotoxic effects of which are probably more extensive than low doses.

Target-Selective Injury to the Renal Medullary Interstitial Cells

With significantly lower doses of BEA (most often 100 mg/kg ip) in Wistar rats (82, 85) there was no evidence of endothelial damage, platelet adhesion, or microvascular leakage using semithin sections; nor was there any change in microvascular filling of the region of papillary necrosis until the lesion was well advanced. The earliest change in the papilla was marked pyknosis of the renal medullary interstitial cells at a time when other cells were normal or near normal. Other investigations using lower doses of BEA and shorter time intervals (15, 16, 19, 82, 85) were unable to show any role for microvasculature injury in the pathogenesis of RPN. The only microvascular effects seen were platelet adhesion well after interstitial necrosis had occurred. The integrity of the medullary microvascular endothelia remained intact even in areas where necrosis had occurred.

BEA Cytotoxicity and RPN

BEA and its analogues 3-bromopropanamine, 2-chloroethanamine, and 2-chloro-*N,N*-dimethylethanamine cause RPN with decreasing potency, which correlates with their alkylating potential and mutagenicity (183). These molecules are all highly reactive as shown by the formation of a series of *N*-carbamate and 2-oxazolidone derivatives (4). The target selectivity of these haloalkylamines has been explained by accumulation in interstitial cells of a direct-acting alkylating species (183). BEA does not, however, affect all cells similarly. It is much more cytotoxic for 3T3 cells and renal medullary interstitial cells than MDCK and HaK cell lines (18), differences that have been related to the coincidence of both lipid droplets (25, 26) and peroxidative enzyme activity (214). The importance of peroxidative metabolism ap-

pears to be supported by the arachidonic-acid-dependent metabolism of 2-bromoethanamine to a cytotoxic metabolite in cultured rat medullary interstitial cell cultures (86). These differences also appear to apply *in vivo,* as BEA has a direct effect on the medullary interstitial cell as the earliest focal lesion (82, 85). It is only subsequently that other fine elements of the medulla such as the loops of Henle, capillaries, and collecting duct cells are affected. The lesion then spreads to include components up to the corticomedullary junction.

Secondary Degenerative Changes Following RPN

Selective damage to the papilla has a profound effect on the kidney and appears to give rise to a series of secondary degenerative changes (8, 15, 228) that are still not fully understood, but parallel closely with the loss of cortical function that occurs in human analgesic abusers (13). There is now evidence that the renal degeneration following BEA-induced RPN has apoptosis as one of its key underlying features (79).

The Modulation of Papillotoxicity

Attempts to modulate RPN could provide additional insight into the mechanism. Thus far these data have provided little definite proof of the underlying processes.

Modulating Prostaglandin Levels

16,16-Dimethyl-PGE_2 (0.75 mg/kg tid po) reduced the incidence of mefenamate-induced RPN (single dose, 200–1,200 mg/kg po) from 63% to 27% in male Sprague-Dawley rats (57). 16,16-Dimethyl-PGE_2 (0.1 and 0.4 mg/kg bid po) also reduced *N*-phenylanthranylic acid-induced RPN (500 mg/kg po for 7 days) in a dose-related response (191). The natural explanation is that 16,16-dimethyl-PGE_2 is cytoprotective and reduces RPN by preventing prostaglandin depletion. PGE_2 does, however, have a number of effects on the kidney, and without more detailed investigations this remains a simplistic explanation rather than proof of the role of PGs.

Renal Sulfhydryl Levels and Drug Metabolism

BEA significantly reduced nonprotein sulfhydryl levels of renal cortex and external medulla but not the papilla. Whereas *N*-acetyl-L-cysteine increased papillary and decreased cortical nonprotein sulfhydryls, a mixture of glutamine, glycine, and cystine produced the opposite effects. Pretreatment with either *N*-acetyl-L-cysteine (6 mmol/kg) or the glutamine, glycine, and cystine mixture protected the cortex, external medulla, and papilla from BEA-induced (1.2 mmol/kg) changes (219, 220). Thus modulating renal GSH affects the potential for BEA to cause RPN, but there is not a clear relationship, as has been the case in liver.

There was a dose-dependent decrease in renal cortical GSH within 1 hr of a single (200, 400, or 600 mg/kg po) diphenylamine dose given to male Syrian hamsters but no changes in outer medullary or papillary GSH. Reducing renal papillary GSH to 29% of basal concentration by prior treatment with L-buthionine sulfoxime (500 mg/kg ip) did not increase the papillotoxicity of a subthreshold dose of diphenylamine (400 mg/kg po). This suggests that diphenylamine-induced RPN in the Syrian hamster is not associated with decreased papillary or outer medullary GSH (134).

Attempts to ameliorate BEA-induced RPN (250 mg/kg, ip) in the Mongolian gerbil (*Meriones unguiculatus*) showed piperonyl butoxide to have no effect (230).

Dimethylsulfoxide

Pretreatment with dimethylsulfoxide (DMSO: 0.5 ml/100 g/day) and diphenylamine (400–800 mg/kg/day) 1 hr later for 3 consecutive days significantly reduced RPN. Similarly, 800 mg diphenylamine in DMSO/kg/day orally for 9 days given to Sprague-Dawley rats caused a focal, apex-limited RPN, but in the absence of DMSO the lesion would have been more substantial. RPN was not observed in female Mongolian gerbils given papillotoxic doses of diphenylamine in DMSO. Thus DMSO protects against diphenylamine-induced RPN, but the mechanism is not clear (136). DMSO is, however, an effective free radical scavenger (129), and this may explain the protective effect. Attempts to ameliorate BEA-induced RPN (250 mg/kg ip) in the Mongolian gerbil (*M. unguiculatus*) showed that DMSO had no effect (230).

Microvascular Changes

Reserpine slowed the development of RPN by its vasodilatory effect on the renal vasculature, not by blocking the endothelial toxicity of BEA in the Mongolian gerbil (*M. unguiculatus*) (230).

The Role of a Concentrating Mechanism and Dehydration, and Water Diuresis

The concentrating process is complex and many aspects are not understood. The central role of the concentrating process in the pathogenesis of RPN is well appreciated by the exacerbation of phenylbutazone (89) and BEA (72) papillotoxicity in animals. In addition, prolonged water imbalance has been used to explain the RPN in rats given formaldehyde in drinking water (230).

Whereas BEA (50 mg iv) caused RPN in heterozygous Brattleboro rats, no lesion developed in homozygous rats (with diabetes insipidus). Administration of vasopressin to homozygous Brattleboro rats fully restored concentrating capacity and the toxic effects of BEA. Decreasing papillary solute concentration by furosemide or increasing urine flow after abrupt withdrawal of vasopressin to homozygous Brattleboro rats did not protect against BEA-induced RPN. Thus, Sabatini (195, 196, 198) concluded that the combination of increased urine flow and decreased papillary solute concentration protects against BEA-induced RPN. Attempts to ameliorate BEA-induced RPN (250 mg/kg ip) in the Mongolian gerbil (*M. unguiculatus*) by diuresis had no effect (230).

There is a general assumption that animals concentrating their urine will be more at risk of developing RPN from any potentially papillotoxic agent (72). There are data to support the exacerbating effects of water deprivation for both phenylbutazone-induced RPN in horses (89) and the BEA-induced lesion in rats (72). There is also evidence that this is not necessarily the case. For

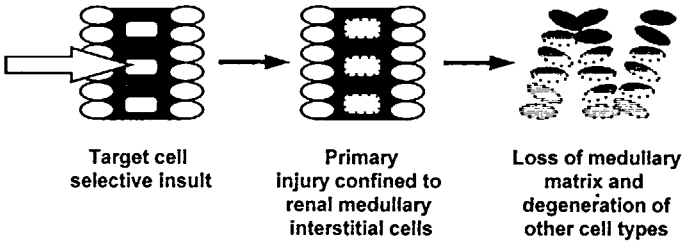

FIG. 3.—Target-selective papillotoxic insult, where the primary injury is confined to renal medullary interstitial cells. This is followed by the loss of the medullary matrix and degeneration of the other medullary cell types.

example, the desert mouse (*Mastomys natalensis*) is much more resistant to BEA than F344 rats (102).

Chronic Degenerative Changes

There is convincing evidence that administration of enalapril (an angiotensin-converting enzyme inhibitor) does reduce the chronic degenerative changes that follow RPN in rats (224) over a 12-mo period.

MECHANISM OF RPN CAUSED BY ANALGESICS AND NSAIDS

Data derived from the BEA and other models of RPN together with what is known about the chronically induced lesion in animals and man have been synthesized into what is now regarded as the "textbook explanation" for RPN (9, 10, 13, 16, 18). The morphological and histochemical changes (8, 15, 19, 82, 85) support distinct pathological changes following a papillotoxic insult. The primary morphological changes occur in the interstitial cells in acute (13, 16, 82, 85), subchronic (92), and chronic (13) papillary necrosis. This is followed by damage to the endothelial cells and loops of Henle, and then collecting duct changes (Fig. 3). While the subtle degenerative changes in the proximal tubule of rats are not central to the papillary lesion, the exfoliation of brush border and proximal tubular cells is an important component of casts that form in the distal nephron. These appear to contribute to marked tubular dilatation, glomerular sclerosis and the loss of effective renal parenchyma (Fig. 4).

The Bioactivation of Papillotoxins in Renal Medullary Interstitial Cells by Peroxidative Enzymes

Phenacetin, *p*-phenetidine, and acetaminophen are converted to reactive intermediates that bind covalently to protein and nucleic acid (3, 12, 45, 52, 115, 156–158, 173, 189, 234). Acetaminophen undergoes oxidative metabolism by prostaglandin H synthase to a reactive quinoneimine that is conjugated to GSH (156, 157). It has been argued that the coadministration of aspirin will serve to deplete GSH (possibly by interfering with the pentose shunt) as a result of which the reactive metabolite of acetaminophen then produces lipid peroxides and arylation of tissue proteins, ultimately resulting in RPN (52). Mohandas et al (158) showed low GSH concentrations, low activities of glutathione reductase, selenium-dependent and selenium-independent glutathione peroxidase and γ-glutamyl transpeptidase in the inner medulla of rabbits. This high level of peroxidative enzymes in the medulla, compared to low levels of detoxifying enzymes, would be expected to make this region of kidney particularly vulnerable to reactive intermediates that were generated locally.

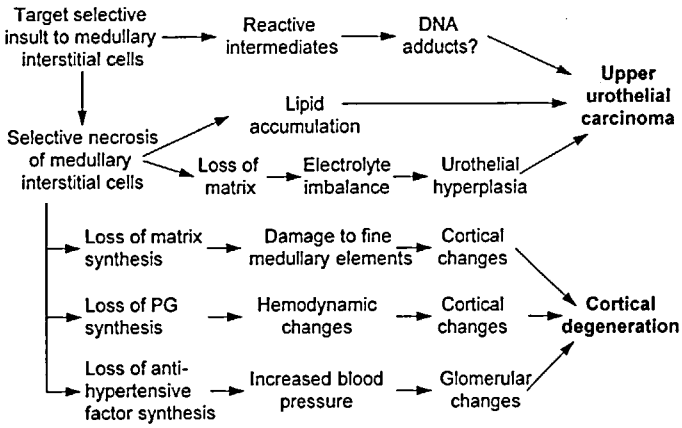

FIG. 4.—Schematic representation of target-selective injury to the medullary interstitial cells and subsequent degenerative cascade and processes that may lead to renal cortical degeneration and upper urothelial carcinoma.

The ubiquitous distribution of prostaglandin synthesis to both the medullary interstitial and collecting duct cells (218) made it most difficult to explain why papillotoxicants affect predominantly the medullary interstitial cells. These cells contain high levels of polyunsaturated fatty acids (25, 26) that would predispose to lipid peroxidation if reactive intermediates were generated locally. The importance of the coincidence of lipid droplets and peroxidative activity (12, 13) in cultured interstitial cells as the basis for their injury has been presented. It is only recently that Khan et al (120) have demonstrated marked species differences in the distribution of cyclooxygenase (COX) I and II isoforms in the medulla of each species. While COX I is constitutive (always present) and COX II is inducible (normally in response to inflammation), both are expressed in the normal kidney. COX I is present in the collecting ducts of all species and COX II in the medullary interstitial cells of all species, except humans. Thus, if the preferential role of either COX I or II can be shown in the metabolic activation of papillotoxic compounds (3, 52, 115, 173, 189), then it will be possible to make a rational risk assessment between species. This differential expression of COX I and II may also explain why the interstitial cells are preferentially affected by some papillotoxins.

Once the interstitial cells are damaged there are degenerative changes in the other "fine elements" of the medulla (Fig. 3) such as the capillaries, loops of Henle, and collecting duct (Fig. 4). If the degree of injury is sufficient, the cortex also undergoes degenerative change, but the factors involved in this cascade of events are poorly understood. Interstitial cells have a key role in the synthesis of prostaglandins, antihypertensive factors, the glycosaminoglycan matrix that surrounds them, and supports the other fine elements of the medulla. The loss of

these cells causes quite marked and profound degenerative changes in the cortex (Fig. 4), which suggests a significant paracrine function (84).

The interstitial cells are highly differentiated and do not repair following necrosis (8, 15, 30, 31, 72, 81–83, 85, 100, 233). This highlights the likely secondary impact of their necrosis, and it is relatively easy to identify a situation in man when exposure to papillotoxins over a number of years will progressively erode the medullary interstitial cell population and produce a similarly slow development of secondary degenerative changes in the cortex. The presence of multiple papillae in humans, as opposed to only one per kidney in most animals, could serve to reduce the clinical consequences of the toxicity of these compounds in humans.

Although this mechanistic explanation (12, 13, 16, 17) is attractive, there are a number of anomalies that suggest that the mechanism is more complex. While phenacetin, acetaminophen, and *p*-phenetidine undergo substantial peroxidative activation (3, 12, 52, 156–158, 173, 189, 234), none has a great propensity to cause RPN in animals (30, 31, 34, 71, 112). This could relate to the extensive hepatic metabolism of these compounds limiting the amount of parent compound that reaches the kidney. Many analgesics and NSAIDs can undergo peroxidative metabolism, but this is not well studied and the propensity to form reactive intermediates in the medulla cannot be confirmed. In addition, chemicals such as L-tri-iodothyronine (119), 2,2-bis(bromomethyl)-1,3-propanediol, nefiracetam, and triethanolamine do not obviously generate such reactive intermediates and therefore do not fit the hypothesis. Furthermore, the RPN caused by some of these compounds (1-naphthol, sulfuryl fluoride) has been after very high doses and it is difficult to identify the usefulness of these data for a mechanistic understanding of the lesion. Long-term exposure to high doses of formaldehyde (222) in drinking water would cause a variety of toxicological changes. Thus more than 1 mechanism probably underlies the development of RPN.

The Relative Papillotoxicity of Analgesics, NSAID and Model Compounds

The problem of putting much of the experimental findings into context is the paucity of comparative data from the same laboratory that allows the papillotoxic potential of each chemical to be estimated. Carlton and Engelhardt (34) have compared the acute papillotoxic effects of several chemicals in Syrian hamsters. 2-Bromoethanamine (75 mg/kg) caused RPN in all animals, whereas 100–400 mg/kg mefenamic acid only affected 40% of animals. A few hamsters given 100–400 mg/kg indomethacin, but none given up to 400 mg/kg acetaminophen or up to 600 mg/kg phenylbutazone, developed renal papillary lesions. This suggests a ranking of the papillotoxic potential in the hamster as 2-bromoethanamine >> mefenamic acid >> indomethacin >> acetaminophen or phenylbutazone. The relevance of this ranking, derived from short-term dosing of laboratory animals to chronic human exposure and the risk of developing papillary necrosis is uncertain.

There are data for horses that suggest the papillotoxic potential of several NSAIDs is phenylbutazone > flunixin > ketoprofen (138). Provided the total dose of phenylbutazone was less than 8.8 mg/kg/day for no more than 4 days or 2–4 mg/kg/day for less than 50 days animals remained clinically normal (69), but there were insufficient data to confirm that such exposure represents a subthreshold papillotoxic level (40). In the absence of water deprivation (87, 88) phenylbutazone is thought to be safe at levels of 8.8 mg/kg/day for 3 mo for horses.

Conclusions and Future Research Directions

Despite all of the resources that have been invested in research, our understanding of medicine-induced and chemically induced RPN remains limited. Many chemicals affect the medulla, but the underlying mechanisms are poorly understood even for those agents that cause an acute or subacute RPN. Some of the uncertainties that underlie the sensitivity of different strains, sexes, or species could be more usefully applied. There are still more questions than answers. For example, despite extensive research on analgesic nephropathy, the lesion cannot be diagnosed noninvasively at an early stage in its development. It has therefore not been possible to identify which analgesic or NSAID has the greatest papillotoxic potential, what the risk factors are, and what combination of analgesics and NSAIDs (and/or other therapeutic agents) may be inappropriate. In the absence of sensitive and selective noninvasive tests to identify early RPN (before secondary cortical degeneration), little progress can be made in epidemiological studies. The underlying mechanism is still controversial. There are no indications as to whether there are risk factors that could predispose individuals to the lesion. While the role of coformulation of caffeine has been presented as a risk factor (36, 95, 98, 179, 180), there is no clear experimental (67, 68, 133, 140) or epidemiological (48, 152, 153) evidence that this is so.

Priorities for future investigations include development of diagnostic techniques to identify the earliest changes, using new technology, such as magnetic resonance spectroscopy and imaging, and molecular biology to help elucidate the pathogenesis of these lesions and the risk to man of exposure to those compounds that affect the medulla. Once the lesion can be identified at an earlier stage it will be possible to better define risk factors. While the concept of direct medullary interstitial cell injury seems to be widely accepted, it is obvious that there are compounds that do not affect these cells. These chemical models should also be studied to understand more about the processes that underlie this "type" of RPN.

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