The New England
Journal of Medicine

Volume 313 NOVEMBER 7, 1985 Number 19

A STUDY OF OCCUPATIONAL EXPOSURE TO ANTINEOPLASTIC DRUGS AND FETAL LOSS IN NURSES

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Abstract In a case–control study, we examined the relation between fetal loss and occupational exposure to antineoplastic drugs in nurses in 17 Finnish hospitals. The pregnancies studied occurred in 1973 through 1980 and were identified using three national sources: the Central Register of Health Care Personnel, the Hospital Discharge Registry, and policlinic data. Each nurse with fetal loss was matched with three nurses who gave birth. Data on health and exposure were obtained by self-administered, mailed questionnaires; a response rate of 87 per cent was achieved after three mailings. A statistically significant association was observed between fetal loss and occupational exposure to antineoplastic drugs during the first trimester of pregnancy: odds ratio = 2.30 (95 per cent confidence interval, 1.20 to 4.39). Analyses suggested associations between fetal loss and cyclophosphamide, doxorubicin, and vincristine, although the independent effect of each individual drug could not be specifically identified, since many nurses reported handling more than one of these agents. The results of this study, combined with existing data on animals and human beings, suggest that caution be exercised in the handling of these valuable drugs. (N Engl J Med 1985; 313: 1173-8.)

ANTINEOPLASTIC drugs as a class include chemically unrelated agents that can inhibit the growth of tumors by disrupting cell growth and killing actively growing cells.[1] This family of drugs includes alkylating agents (e.g., cyclophosphamide), antimetabolites (e.g., fluorouracil), spindle poisons (e.g., vincristine), antibiotics (e.g., doxorubicin), and hormones (e.g., diethylstilbestrol).[2] Embryos and fetuses, which have many growing cells, are especially susceptible to the toxic effects of these agents.

Many antineoplastic drugs have been reported to be carcinogenic, mutagenic, and teratogenic. In cancer patients, therapeutic doses of these drugs have been associated with subsequent cancers[3,4] and with increased numbers of sister-chromatid exchanges and chromosomal aberrations.[5-8] Case reports have described the birth of malformed infants to patients treated with these drugs during pregnancy.[9-12] The usefulness of these reports is limited because of the small numbers, the preexisting illnesses of the patients, the lack of comparative data, variations in exposure, and exposure to several agents. Therapeutic doses to patients are much higher than the amounts received by hospital personnel through inhalation or skin absorption.

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Several antineoplastic drugs (cyclophosphamide, diethylstilbestrol, fluorouracil, methotrexate, and vincristine) have been reported to have teratogenic and mutagenic in vivo effects in several animal species.[13,14] Cyclophosphamide is frequently used as a positive control in studies of teratogenesis (Hardin B: personal communication).

Studies of occupational exposures have shown detectable levels of antineoplastic drugs in the air of hospital units with no hoods.[15] Historically, nurses have prepared doses in areas without hoods. Also, hood use does not guarantee reduced ambient levels. Although vertical laminar-flow hoods decrease worker exposure, horizontal laminar-flow hoods, which are designed to prevent contamination of medicines, do not prevent such exposure.[16] Mutagenic agents have been found in the urine of hospital personnel working with antineoplastic drugs in areas with horizontal laminar-flow hoods or without hoods, but have not been found in situations in which vertical laminar-flow hoods are used.[17-22]

Pharmacy personnel and nurses working with antineoplastic drugs have been reported to have increased sister-chromatid exchanges,[23-25] chromosomal gaps,[25] and mutagenic agents in their urine,[17-20] although one study of sister-chromatid exchanges did not report such changes.[26] Studies of animal cell lines have also found increased sister-chromatid exchanges,[27,28] chromosomal breakage,[28] and mutagenicity.[29]

A recent Finnish case–control study of malformations in births and fetal loss in nurses exposed to anes-

thetic gases also collected data on other hospital exposures.[30] It was noted that first-trimester exposure to antineoplastic drugs was significantly more common among nurses who gave birth to malformed infants than among those who delivered normal infants. This incidental finding suggested the need for a study specifically designed to examine the effects of these drugs on the outcome of pregnancy. We report on a case–control study of the possible association between fetal loss and exposure to antineoplastic drugs among nurses.

Methods

Population Identification

The study population, located in Finland, was identified through three sources: the Central Registry of Health Care Personnel, which included all health care personnel in Finland; the Hospital Discharge Registry, which contained data on all hospitalizations in Finland from 1973 through 1980; and policlinic data for 1973 through 1980.

All nurses employed in Finnish hospitals are listed in the Central Registry of Health Care Personnel, started in 1930 and first computerized in 1979. Nurses are registered upon graduation, and data are updated by employers at the times of hiring and of termination. The nurse is responsible for providing further information at other times. Registry computer files from 1979 and 1980 identified nurses employed in hospitals that reported high usage of antineoplastic drugs to the Finnish Institute for Occupational Health in 1979. "High usage" was defined as the use of at least 100 g of cyclophosphamide (the most commonly given antineoplastic drug in Finland) per year or at least 200 g of all antineoplastic drugs per year. The potential study group was further restricted to female nurses who were 40 or younger in 1980 and were registered as working in wards in which antineoplastic drugs may have been used. The study pregnancies did not necessarily occur in the wards identified in 1979 to 1980.

These nurses were linked by personal identifier to their pregnancies listed in the Hospital Discharge Registry and policlinic data. Pregnancies were restricted to *International Classification of Diseases* (ICD, 8th revision) codes 643 and 645 for fetal loss, and 650 through 662 for births. Multiple listings for the same pregnancy were removed, and the time of the last menstrual period was estimated.[31] A pregnancy qualified for study if it occurred after graduation and if the last menstrual period was between October 1972 and March 1980. This second restriction allowed births and fetal losses among women with the same last menstrual period to be studied, even if the lengths of gestation were different.

Statistical problems can result from examination of nonindependent events (multiple pregnancies in one woman)[32]; therefore, only one pregnancy per nurse was included in the final study group. If a woman had more than one fetal loss during the study period, one loss was randomly selected. For potential controls, one birth was randomly selected for each remaining woman without a recorded fetal loss. Since the ICD codes refer to the mother's hospitalization, both live births and stillbirths were included, but the control mothers did not have registered fetal losses during the study period. However, some fetal losses may have occurred before the study or may not have been registered because of errors in the system or because the nurse did not seek medical care.

Three controls from the same hospital were selected for each case, using the closest matches[33] based on the age of the mother at her last menstrual period ±2 years. Three matches were obtained for all but nine fetal losses.

Data Collection

Brief, self-administered questionnaires were mailed to the participants to obtain data on health status, personal habits (alcohol use and smoking), and lifetime reproductive and work histories. Data on medications; illness; injury; personal habits; and exposure to antineoplastic drugs, x-rays, anesthetic gases, and ethylene oxide were collected in relation to the study pregnancy. A two-year period that encompassed the study pregnancy was specified and the respondents were asked to provide data on all pregnancies during that time.

Returned questionnaires were checked for internal consistency and completeness. Photocopies of incomplete questionnaires were returned to the nurses with a request to respond to any unanswered questions.

Data Analysis

The data were analyzed with conditional logistic regression because the controls were individually matched and this analysis maintains the matches.[34] This procedure also allows an unequal number of controls for each case, which resulted from the response patterns in the study group.

Questionnaire data on hospital exposures were collected for three months before the last menstrual period and each month during pregnancy. A minimum exposure frequency of once a week separated the regularly exposed nurses from those with superficial exposure. Those exposed less than once per week were considered unexposed, a definition that tends to underestimate any associated risk. In addition, only nurses who prepared antineoplastic drug doses were considered exposed — an approach that excluded only three nurses. Since few nurses were exposed to ethylene oxide, this exposure was excluded from analyses. Two exposure models were used: first-trimester exposure (defined as working for two months or longer during the interval from one month before the last menstrual period through three months afterward), and cumulative exposure (years of exposure before the last menstrual period). Cumulative occupational exposures were used to assess long-term effects (e.g., mutagenic effects on germ cells).

The following variables were included in initial models, along with occupational exposures, on the basis of a priori evidence: smoking, alcohol use, health conditions, and medications (including contraceptive failures) in the first trimester of pregnancy; gravidity; and prior history of fetal loss and induced abortion.[9,35-39] Failures of oral contraceptives and intrauterine devices were combined because of small numbers and all "other" contraceptive methods were combined. The time of the pregnancy within the study period (before or after January 1, 1977) was tested to examine recall differences. Risk factors, selected on the basis of a priori evidence, were tested for inclusion at $P = 0.10$; other factors, such as potential effect modifiers (interaction terms), were tested at $P = 0.05$.

Results

Of the 650 study subjects, 87.4 per cent responded (83.2 per cent of the cases and 88.8 per cent of the controls, Table 1). The study pregnancy was reported by 89.2 per cent of the case respondents and 98.1 per cent of the control respondents.

Table 1. Response Status of the Population.

	Cases (Fetal Loss)	Controls (Birth)	Total
	no. of subjects (%)		
Total population	167 (100.0)	483 (100.0)	650 (100.0)
Respondents	139 (83.2)	429 (88.8)	568 (87.4)
Reported pregnancy of interest (% of total)	124 (74.3)	421 (87.2)	545 (83.8)
Nonrespondents	28 (16.8)	54 (11.2)	82 (12.6)
Reason for no response			
No answer	21 (12.6)	46 (9.5)	67 (10.3)
Refusal	6 (3.6)	3 (0.6)	9 (1.3)
Other (e.g., could not locate, out of country)	1 (0.6)	5 (1.0)	6 (0.9)

Table 2. Description of the Population Used in the Final Analysis.*

	CASES	CONTROLS
No. of observations	124	321
Mother's age at conception (mean ±S.D.)	27.6±3.6	27.4±3.4
Gravidity		
Mean ±S.D.	2.0±1.2	1.7±0.8
Pregnancies at gravida 1	49 (39.5)	164 (51.1)
Previous fetal losses	24 (19.4)	28 (8.7)
Previous induced abortions	9 (7.3)	12 (3.7)
Characteristics of first trimester of pregnancy		
Alcohol drinking		
< Once/mo	97 (78.2)	277 (86.3)
1–3 times/mo	18 (14.5)	39 (12.1)
≥ Once/wk	9 (7.3)	5 (1.6)
Cigarette smoking		
Cigarette smokers	17 (13.7)	41 (12.8)
Cigarettes/day (mean ±S.D.)	6.6±6.1	6.7±4.3
Illness	16 (12.9)	38 (11.8)
Any contraception at conception	17 (13.7)	10 (3.1)
Oral contraceptives	3 (2.4)	0
Intrauterine device	8 (6.5)	4 (1.2)
Other	6 (4.8)	6 (1.9)
Employed as a nurse	114 (91.9)	301 (93.7)
Medication	13 (10.5)	35 (10.9)

*Values are numbers of subjects with percentages in parentheses except where otherwise indicated.

The final analysis was limited to fetal losses with detailed pregnancy data (74.3 per cent) and their matched controls, which are referred to as case–control sets. Table 2 describes the major demographic and pregnancy-related characteristics used in the analyses, and Table 3 describes the occupational exposures of the nurses during the study pregnancy.

Early stages of modeling included both cumulative and first-trimester exposures. Cumulative exposure measures were not associated with fetal loss; therefore, this report is restricted to analysis of first-trimester occupational exposures.

The final model (Table 4) is consistent with an association between antineoplastic drugs and fetal loss (odds ratio = 2.30, 95 per cent confidence interval = 1.20 to 4.39). Other factors associated with fetal loss included prior fetal loss or induced abortion, alcohol consumption, and failures of oral contraceptives or intrauterine devices (Table 4). At this stage, effect modification was examined for the factors described in Table 4. Prior fetal loss was an effect modifier for both antineoplastic drugs and x-ray exposure during the first trimester: the odds ratio for both exposures for women without prior losses was significantly increased (antineoplastic drugs: odds ratio = 3.30, confidence interval = 1.62 to 6.74; x-ray exposure: odds ratio = 4.50, confidence interval = 1.63 to 12.47); the odds ratios for women with prior losses (n = 52) were not different from 1.

After the initial analysis was completed, the variable for "all antineoplastic drugs" was removed from the analysis, and individual drugs reported by at least 10 women (cyclophosphamide, doxorubicin, fluorouracil, and vincristine) were tested one at a time. All these drugs except fluorouracil were significantly associated with fetal loss (Table 5). However, when all four drugs were entered into the same model, none of them were significantly related to fetal loss. Many nurses were exposed to several of these drugs during the first trimester (Fig. 1), and the likelihood of exposure to more than one was high ($P<0.01$ correlation) and thus separation of the effects of individual drugs was not possible.

Mean gestation was slightly shorter for fetal losses among exposed women, as compared with fetal losses among unexposed women: 11.4 versus 13.0 weeks

Table 3. Description of Nurses' Occupational Exposures at the Time of the Study Pregnancy.*

	CASES	CONTROLS
No. of observations	124	321
Antineoplastic drugs		
Exposed first trimester	18 (14.5)	28 (8.7)
1–5 times/wk	14 (11.3)	21 (6.5)
6–10 times/wk	1 (0.8)	3 (0.9)
>10 times/wk	3 (2.4)	4 (1.2)
Most frequently reported drugs		
Doxorubicin	7 (5.6)	7 (2.2)
Cyclophosphamide	13 (10.5)	19 (5.9)
Fluorouracil	5 (4.0)	12 (3.7)
Vincristine	12 (9.7)	17 (5.3)
Long-term exposure		
No. reporting exposure	38 (30.6)	93 (29.0)
Years of >1/wk (mean ±S.D.)	2.26±2.34	2.54±2.44
Anesthetic gases		
Exposed first trimester	9 (7.3)	14 (4.4)
Chronic exposure		
No. reporting exposure	21 (16.9)	39 (12.1)
Years of >1/wk (mean ±S.D.)	2.17±2.33	2.04±2.42
X-rays		
Exposed first trimester	13 (10.5)	14 (4.4)
Chronic exposure		
No. reporting exposure	33 (26.6)	82 (25.5)
Years of >1/wk (mean ±S.D.)	2.45±2.08	2.56±2.40
Ethylene oxide		
Exposed first trimester	0 (0.0)	1 (0.3)
Chronic exposure		
No. reporting exposure	3 (2.4)	7 (2.2)
Years of >1/wk (mean ±S.D.)	0.60±0.56	2.07±2.64

*Values are numbers of subjects with percentages in parentheses except where otherwise indicated.

Table 4. Conditional Logistic Analysis of Fetal Losses among Nurses.

	Beta (S.E.)	Odds Ratio (95% Confidence Interval)	P Value
First-trimester exposures			
Antineoplastic drugs	0.83 (0.33)	2.30 (1.20–4.39)	0.01
Anesthetic gases	−0.04 (0.50)	0.96 (0.36–2.59)	0.94
X-rays	0.82 (0.45)	2.27 (0.94–5.47)	0.07
Any previous fetal loss	0.99 (0.34)	2.69 (1.39–5.21)	0.003
Any previous induced abortion	1.02 (0.50)	2.77 (1.04–7.37)	0.04
Alcohol drinking			
<Once/mo		1.00	
1–3 times/mo	0.17 (0.33)	1.19 (0.62–2.26)	0.60
≥Once/wk	1.65 (0.57)	5.18 (1.69–15.88)	0.004
Use of contraception at conception (OC and IUD)*	1.89 (0.62)	6.60 (3.56–22.16)	0.002

*OC denotes oral contraceptives, and IUD intrauterine device.

for losses at the hospital and 9.1 versus 9.5 weeks for losses at the policlinics (P>0.05).

Discussion

This study found that women who experienced fetal loss were more than twice as likely to have had first-trimester exposure to antineoplastic drugs as women who gave birth were (odds ratio = 2.30). An association between fetal loss and cumulative exposure to antineoplastic drugs was not observed. The odds ratio for women without prior fetal losses was higher (odds ratio = 3.30), whereas no association was observed for women with prior fetal loss. The women with prior loss probably had an inherently higher background risk of fetal loss,[39] perhaps because of a persistent maternal (or paternal) factor, so the incremental risk due to occupational exposure should have been less in comparison with the background risk. A similar pattern was found for smoking and prior fetal loss in a study by Kline et al.[38]

As in any study relying on questionnaire data, differential response rates between the cases and controls could have affected the findings. However, reassurance on this point was provided when two variables from the registries that were available for all study members (the estimated date of the last menstrual period and the woman's age at that time) were compared in terms of respondent status within the case and control groups. The mean values for these groups were not significantly different. Some study pregnancies were identified by the registries but not reported by the respondents (Table 1). Unreported events may have resulted from differences between the registry data and the women's recall of dates of pregnancies, or from errors in recall (especially for fetal loss)[40,41] or registration. Previous research has found almost perfect agreement on women's recall of dates for births[40] and good agreement (within one year) on recall of fetal losses.[40,41] Recall was most inaccurate for fetal loss occurring early in gestation,[41] and thus for events less likely to be identified in the data sources used in this study. In the current study, eight control births either were not reported by the women or the dates of the births were very different from those recorded by the registries. These data suggest that some of the nonmatches in this study were due to errors in registration.[31] A disproportionate amount of registered fetal losses (10.8 per cent), as compared with births (1.9 per cent), were not mentioned in the questionnaire. Inconsistent reports suggest errors both in registration and in recall or reporting of the women. Recall of exposure could vary with the time since pregnancy; a significant correlation was not observed between reported antineoplastic drug exposure and the timing of the pregnancy within the study period.

Another analysis examined results, including data for the study pregnancy, when the detailed data were not reported in returned questionnaires. The number of matched case–control sets rose from 124 to 139 (83.2 per cent of the total sets). Detailed pregnancy data were used when possible, and data for unreported pregnancies were estimated from information on lifetime employment and personal habits. All variables in the final model, except for contraceptive use, had alternative sources of data from the lifetime histories in the questionnaire and were present in the analysis. The results for antineoplastic-drug and x-ray exposure were essentially the same; the odds ratio for anesthetic gases (1.75; confidence interval = 0.72 to 4.29) changed direction, but was still not significant.

Detailed personnel records and industrial data on exposures of individual nurses did not exist, and nurses working in the same hospital units did not have the same likelihood of exposure. For all exposures at work, data were obtained on the month of pregnancy and self-reported frequency of exposure in episodes per week. Although data on hood and glove use were not obtained, such use was not common during the study period. The frequency data could incorporate many degrees of exposure in that a nurse could be preparing one or many doses of antineoplastic drugs per episode. Nurses working with antineoplastic drugs less than once a week would probably not prepare a

Table 5. Results of Conditional Logistic Regression Examining Antineoplastic Drugs One at a Time and All at Once.*

	Odds Ratio (95% Confidence Limits)	
	Drugs Examined One at a Time	Drugs Entered in the Same Model
Doxorubicin	3.96 (1.31–11.97)	2.45 (0.61–9.78)
Cyclophosphamide	2.66 (1.25–5.71)	1.77 (0.47–6.69)
Fluorouracil	1.70 (0.55–5.21)	0.62 (0.15–2.53)
Vincristine	2.46 (1.13–5.37)	1.50 (0.51–4.42)

*These analyses included other nursing exposures, previous fetal loss and induced abortion, alcohol consumption, and use of contraception at conception.

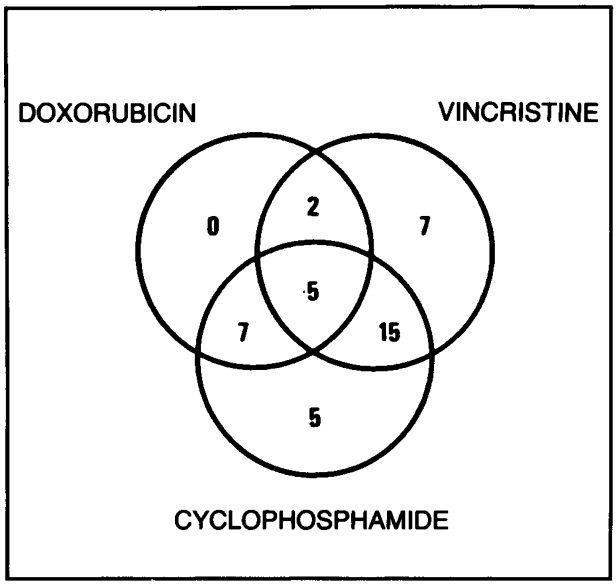

Figure 1. Diagram of Occupational Exposure to Antineoplastic Drugs.

Forty-one nurses reported exposure to antineoplastic drugs. Of the 14 (34.1 per cent) exposed to doxorubicin, none were free of exposure to the other two drugs. Of the 32 (78.0 per cent) exposed to cyclophosphamide, 5 (16.1 per cent) were not exposed to the other drugs, and of the 29 (70.7 per cent) exposed to vincristine, 7 (24.1 per cent) were not exposed to the other drugs.

large number of doses at a given time, and thus their exposures would be limited. Because of the small number who reported very frequent exposure (more than five times a week), low- and high-exposure pregnancies could not be compared.

If the outcome of pregnancy produced a bias in recall of pregnancy-related exposures, it was probably minor. If such a bias were present, an elevated odds ratio would be expected for anesthetic gases, since several reports have linked this exposure to fetal loss.[42,43] However, the odds ratio for anesthetic gases was low in the primary analysis (Table 4).

The risk estimate for antineoplastic drugs may be underestimated. Not all the controls delivered normal infants. One stillbirth occurred, and the nurses reported that 15 of the live infants had deformities. In these cases, 26.7 per cent of the mothers reported antineoplastic drug exposure during the first trimester of pregnancy, as compared with 8.7 per cent exposure for all births.

The observed association between fetal loss and exposure to antineoplastic drugs is not surprising, given the mechanisms of action of these drugs and existing data on animals and human beings. The results of this study, along with existing data, suggest that caution be exercised in the handling of these useful agents.

We are indebted to Markku Sallmen, Pentti Kyyronen, and Dr. Helena Taskinen of the Finnish Institute of Occupational Health for help during the data-collection phase of this project, and to Dr. Philip Landrigan of the National Institute for Occupational Safety and Health for his support during the analysis.

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ALDOSTERONE-RECEPTOR DEFICIENCY IN PSEUDOHYPOALDOSTERONISM

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Abstract Pseudohypoaldosteronism, a syndrome characterized by salt wasting and failure to thrive, usually presents in infancy as high urinary levels of sodium despite hyponatremia, hyperkalemia, hyperreninemia, and elevated aldosterone levels. We have investigated this syndrome for the possibility of abnormal Type I or "mineralocorticoid-like" receptors, which have intrinsic steroid specificity indistinguishable from that of renal mineralocorticoid receptors and are found in many tissues and cells, including mononuclear leukocytes. We have studied three patients with pseudohypoaldosteronism: the 28-year-old index case in Melbourne (Patient 1) and two siblings in Munich, eight and two years of age (Patients 2 and 3); clinically, Patient 3 had a less severe case than his sister. Percoll-separated control monocytes bound [^{3}H]aldosterone with high affinity (K_d~3 nM) and limited capacity (150 to 600 sites per cell). On repeated examination, no [^{3}H]aldosterone binding was found in monocytes from Patients 1 and 2; in Patient 3, the levels were 62 sites per cell, more than 2 S.D. below those of the control. Levels in the parents of the Munich patients (first cousins) were normal. It appears that pseudohypoaldosteronism is caused by a Type I receptor defect, that the defect may be complete or partial, that transmission may be autosomal recessive, and that the study of patients with pseudohypoaldosteronism may indicate physiologic roles for Type I receptors in nonepithelial tissues. (N Engl J Med 1985; 313:1178-81.)

IN 1958 Cheek and Perry[1] described a male infant in whom renal salt wasting was associated with normal renal and adrenal function. The condition was corrected by sodium chloride supplementation but was refractory to exogenous mineralocorticoid without added salt. Since the ratio of urinary sodium to potassium was not altered by the administration of desoxycorticosterone, Cheek and Perry suggested that the pathogenesis of the condition was a defective renal tubular response to mineralocorticoids.

More than 25 years later, a recent review[2] concluded as follows:

> Little has been learned about the pathophysiology of this disorder since the original report of Cheek and Perry. It appears likely that these patients represent an end-organ defect in aldosterone responsiveness. In order to establish whether this is due to a defect in Type I receptors, a post-receptor defect, or a reduced number of receptors, it would be necessary to obtain sufficient tissue, such as kidney, sweat glands, or bowel, to conduct receptor studies. At this time, this is not feasible.

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Supported by the Alexander von Humboldt Stiftung (Dr. Armanini); by grants (Ku 428/2-1, to Dr. Kuhnle, and Do 273/1-1, to Dr. Dorr) from the Deutsche Forschungsgemeinschaft; by a grant (82,004.1) from the Wilhelm Sander Stiftung (Dr. Strasser); and by the National Health and Medical Research Council of Australia (Dr. Funder).

In early studies on mineralocorticoid receptors,[3,4] aldosterone was shown to bind to two classes of limited-capacity sites in rat-kidney preparations. Those with a higher affinity for aldosterone — the putative mineralocorticoid-effector sites — were termed "Type I" receptors. Those with a lower affinity for aldosterone were termed "Type II" sites, and were subsequently shown to be glucocorticoid receptors.[5] It was also subsequently shown that Type I sites are widely distributed in various cells and tissues — for example, the hippocampus[6] and mammary gland[7] — that are not classic mineralocorticoid target tissues. The physiologic role of Type I receptors in such tissues remains to be established.

Though most studies of Type I receptors have been in rat tissues, comparable sites have been demonstrated in the human kidney.[8,9] Very recently, Type I sites