Bioassay of Different Formulations of Aspirin by Means of Human EEG

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Several commercial formulations of aspirin were compared for potency and duration of action. Quantitative human EEG was employed as the bioassay. The effects of aspirin as determined by this technique are highly reproducible and dose-related, resembling qualitatively the effects of common "antianxiety" drugs. The results show that differences in formulation lead to differences in pharmacological effect.

INNUMERABLE claims appear continually about the relative efficacy and speed of action of different formulations of aspirin. Careful evaluation of these is important, because aspirin is probably the most heavily consumed of all drugs. Serum salicylate concentrations give information about absorption from the gastrointestinal tract into the blood stream, but are not directly informative about the time course of the concentrations of aspirin at its pharmacological sites of action. The latter can be determined only by means of some kind of bioassay, preferably in humans, since man is the species of interest where the consumption of this drug is concerned. Aspirin does not reliably influence pain thresholds or other varieties of experimentally induced pain which can be employed in humans, although some information has been gained from dogs by means of the intra-arterial bradykinin technique of Lim (1). Clinical pain can be demonstrably influenced by aspirin, but not with sufficient precision to differentiate among various formulations. Therefore, a new approach has been needed to delineate with sensitivity and accuracy the time course and potencies of aspirin preparations in their pharmacological actions in humans.

The authors have reported previously (2) that aspirin has characteristic, highly reproducible effects upon the quantitative electroencephalograms (EEG) of normal human volunteers. The present report describes the results obtained when the earlier trials were replicated with larger numbers of subjects given varying doses and when different commercial preparations of aspirin were compared. Some related drugs were again studied concurrently for comparison.

METHODS

The method employed quantitative analysis of the EEG, recorded "monopolarly" from the left occipital area. Fifty-four subjects, 31 male reformatory inmates and 16 male and 7 female members of the laboratory staff, ages 18 to 30, took part in a total of 279 experimental sessions. During the first hour of a standard 7-hr. session, the electrodes were affixed and a 10-min. recording was obtained. Immediately after this, the drug (or placebo) was administered orally. Thereafter, 10-min. recordings were made every hour for 6 hr. During all recordings the subjects were reclining with eyes closed in a dimly lighted, quiet room. Between recordings, the subjects were free to move around or to sit and read in a lounge adjoining the EEG recording room.

Quantitation of the EEG was performed with an electronic integrator (3, 4). This device transforms the complex EEG signals into pulses which are recorded concomitantly with the direct tracings. The number of pulses for any given time period is directly proportional to the cumulated electrical energy. Calibration is by the application of known energy constants, and the values obtained therefore can be related to fixed standards. The basic time-unit chosen for analysis was 20 sec. (that is, 2 pages of standard EEG); thus any 10-min. recording yielded 30 successive measurements. The number of pulses during each 20-sec. period was automatically totalized and printed on paper tape. Artifacts were easily removed from the counts since each set of numbers could be related to the page of direct recording from which it was obtained.

All the corresponding values from each predrug and postdrug run, as obtained from all subjects involved in each study, were averaged, and the mean energy content (MEC) for the group was thus determined. Observed differences in MEC were tested for statistical significance by means of the t test. In addition, a careful analysis of variability within subjects was performed for each time period. It has been previously shown (5) that this parameter of quantitated EEG data is highly informative, not only for the detection and characterization of drug effects, but also for baseline features. For example, it has been found that male chronic schizophrenics have much less EEG variability than nonpsychotics (5). Statistical significances of the differences in variability were determined from F ratios. The within-subject variabilities are shown in the tables as coefficients of variation (CV). This is to allow averaging of the variability in the records of subjects who have different MEC values.

Since the drugs were ordinary commercial preparations obtained locally, it was not always possible to have identical-appearing placebo and active medication tablets. However, in most cases the tablets were similar in form and color. Strict double-blind design was followed in all other respects. Cross-over of subjects from one study to another served to minimize possible biases.

RESULTS

Table I gives the results obtained with effective doses of the different forms of commercially avail-

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TABLE I—EFFECTS OF VARIOUS FORMS OF ASPIRIN (AND OF PLACEBO) ON THE ELECTRICAL ENERGY LEVELS AND VARIABILITY OF THE LEFT OCCIPITAL EEG IN NORMAL SUBJECTS

Drug, Dose, and No. of Subjects		Predrug Control	hr. Following Drug Administration[d] 1	2	3	4	5	6
Generic aspirin	MEC[a]	61	63	59	53*	54*	65	64
1.0 Gm., N[c] = 20	CV[b]	20	24	26	40*	40*	27	27
Brand aspirin	MEC	70	68	60*	62*	63*	72	71
1.0 Gm., N = 10	CV	25	27	34*	41*	27	29	30
Generic buffered aspirin	MEC	76	64	67	56*	66	72	63
1.0 Gm., N = 10	CV	25	39*	37*	43*	37*	41*	31*
Brand buffered aspirin	MEC	49	45	38*	38*	41*	36*	41*
1.0 Gm., N = 10	CV	27	35	43*	42*	42*	38*	37*
Brand buffered aspirin	MEC	83	75	73*	74*	75	86	82
660 mg., N = 10	CV	27	32	35*	32	34	29	34
Effervescent aspirin	MEC	84	74	72*	72*	96	95	81
1.0 Gm., N = 10	CV	22	33*	34*	36*	23	24	33*
Placebo	MEC	62	63	65	65	65	62	67
N = 20	CV	18	21	23	26	23	25	19

[a] MEC, mean integrated energy levels of the EEG. [b] CV, coefficient of variation (standard deviation × 100/mean). [c] N, number of subjects. (The actual number of measurements used to compute the variance was ($N \times 30$) − N; thus 580 in the case of generic aspirin.) [d] The asterisk denotes statistically significant departure from control levels (t tests for MEC, F ratios for variances) at the 0.05 level or better.

able aspirin. In most of the experimental trials the effective dose was 3 tablets or about 1.0 Gm. As can be seen, two significant changes occurred after all the dosage forms, namely, a decrease in MEC and an increase in CV. This is the characteristic effect of aspirin. The times of onset and peak of action were different, but the dual trend was present in all cases. With brand aspirin, the shifts occurred sooner and lasted longer than with generic aspirin. The most pronounced effects were observed following the administration of brand buffered aspirin. With this drug a significant dose-response relationship was shown in that 2 tablets had a relatively smaller and shorter-lasting action than 3 tablets. This is illustrated in Fig. 1. Placebo did not produce significant changes. The threshold dose for significant EEG effects was found to be 2 tablets; 1 tablet (330 mg.) was ineffective.

It will be noticed that the initial levels of the MEC varied in magnitude among the different groups. This is due to random assortment of the subjects with high or low amplitude of the so-called "α-waves." On the other hand, the coefficient of variation was found to be constant (within the limits of statistical variation) from group to group.

From the standpoint of visual inspection of records, the changes in MEC and CV corresponded to what is sometimes called "EEG drowsiness," *i.e.*, flattening of the amplitude of α-waves with

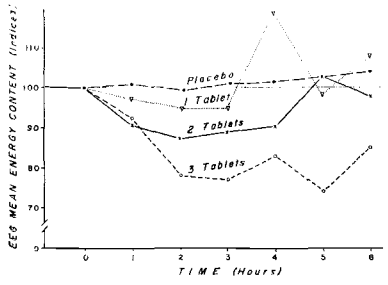

Fig. 1—Dose-response curves of the effect of brand buffered aspirin on the quantitative EEG of 4 groups of 10 subjects each.

sporadic occurrence of waves corresponding to light sleep and even some high amplitude slow waves.

Of the other antipyretic compounds studied, sodium salicylate, salicylamide, and acetaminophen had negligible effects (Table II). Significant increases in the CV occurred 1, 2, 4, 5, and 6 hr. after phenacetin, but there was no significant shift in the MEC. The lack of effect of sodium salicylate might be a result of its slow rate of absorption (6), since the time course of the EEG changes after aspirin correlates with previously reported curves of the serum levels of salicylates following oral administration (7). Another kind of analgesic, codeine, was studied at 2 dose levels, 32 and 64 mg. orally. After 32 mg., a dose which is commonly employed clinically for analgesic effect, no changes in the EEG were detected (Table II). The larger dose of codeine, 64 mg., caused significant increases in variability with no alteration in the MEC; thus it resembled phenacetin in effect but was unlike aspirin.

When the aspirin data are compared with those obtained from other classes of compounds, a striking resemblance to the effects (8) of those drugs which are variously called "minor tranquilizers" or "anti-anxiety" drugs is observed. In Table III, data from experiments performed with normal subjects given 3 such agents are presented. Although differences in time course and extent of change occurred, the characteristic decrease in MEC and increase in CV were present nonetheless. It should be emphasized that these changes are characteristic of the anti-anxiety class of drugs only; neither stimulants nor potent depressants nor any other kind of psychotropic agent yet tested produces the same kind of action. For example, stimulants decrease both the MEC and the variability of the EEG; major tranquilizers increase the MEC and decrease the CV. It would thus appear that in normal subjects aspirin produces changes in occipital electrical activity very similar to those caused by anti-anxiety agents. Since this effect is not mimicked by a more potent analgesic, codeine, it is probably not related to analgesia *per se*.

DISCUSSION

The drugs used in these studies were purchased

TABLE II—EFFECTS OF AN ANALGESIC (CODEINE) AND OF DRUGS RELATED TO ASPIRIN ON THE ELECTRICAL ENERGY LEVELS AND VARIABILITY OF THE LEFT OCCIPITAL EEG IN NORMAL SUBJECTS[a]

Drug, Dose and No. of Subjects		Predrug Control	hr. Following Drug Administration 1	2	3	4	5	6
Codeine, 32 mg.	MEC	62	66	67	69	67	68	68
$N = 10$	CV	18	19	19	22	19	18	24
Codeine, 64 mg.	MEC	76	75	75	72	78	72	77
$N = 10$	CV	19	28*	31*	26*	24	30*	25
Sodium salicylate	MEC	68	73	70	70	71	70	65
1.3 Gm., $N = 10$	CV	17	15	23	18	17	21	22
Phenacetin, 1.0 Gm.	MEC	60	62	62	61	58	63	59
$N = 21$	CV	21	33*	38*	26	48*	32*	31*
Salicylamide, 1.0 Gm.	MEC	38	47*	42	39	42	38	43
$N = 13$	CV	21	25	25	20	20	21	21
Acetaminophen	MEC	63	63	64	63	63	62	59
1.0 Gm., $N = 10$	CV	18	20	24	23	19	29*	25

[a] See Table I for explanation of the symbols used.

TABLE III—EFFECTS OF ANTIANXIETY DRUGS (AND PLACEBO CONTROLS) ON THE ELECTRICAL ENERGY LEVELS AND VARIABILITY OF THE LEFT OCCIPITAL EEG IN NORMAL SUBJECTS[a]

Drug, Dose and No. of Subjects		Predrug Control	hr. Following Drug Administration 1	2	3	4	5	6
Chlordiazepoxide	MEC	43	46	43	34*	33*	34*	32*
20 mg., $N = 9$	CV	19	23	28*	23	24	19	33*
Meprobamate	MEC	60	58	43*	46*	53	54	64
800 mg., $N = 10$	CV	19	29*	32*	38*	47*	56*	39*
Phenobarbital	MEC	71	60*	63	65	56*	51*	61
20 mg., $N = 20$	CV	19	29*	22	19	25*	25*	27*
Placebo	MEC	58	57	56	64	65	57	61
$N = 10$	CV	28	25	27	32	29	35	19*

[a] See Table I for explanation of the symbols used.

from local commercial sources. The identification of the brands used is not germane to this scientific report. The authors believe that this study indicates that careful pharmaceutical formulation produces a greater "aspirin effect" on the human brain. The careful selection of buffers and binders is important. It is well known that acetylsalicylic acid in the presence of moisture undegoes hydrolysis to salicylic and acetic acids. The question can be raised as to whether the reported differences between "generic" and "brand" aspirin are due to the relative commercial turnover of these different preparations, in other words, to the time duration between production and consumption; this could be faster in the case of "brand" products, with the result of a higher concentration of acetylsalicylic acid. No attempt was made to determine the extent to which this assumed difference could have biased the data, primarily because the aim was to reproduce as nearly as possible the conditions under which the different forms of aspirin are used in everyday life. Various commercial firms claim that bottled aspirin will remain stable for many years. This is probably true, since no date limit is required for its medical use.

The results of this study raise questions about the extent to which people take aspirin for effects other than relief of pain, fever, or inflammation, and their reasons for doing so. Because the EEG effects of aspirin are similar to those of compounds such as phenobarbital, meprobamate, and chlordiazepoxide, it is possible that other effects may also be similar and may thus account for the popularity of aspirin which, unlike the other drugs, can be purchased over the counter and taken without any pejorative connotations. Even if aspirin is not obviously effective in marked anxiety, it may be sufficiently active to relieve the lesser anxieties and tensions which occur in everyday life. Some support for this has appeared in two reports (9, 10) of studies which employed psychological rating scales to assess the effects of aspirin in groups of hospital attendants, nurses, and other employees. Significant reductions in "depression" and "fearfulness" occurred after aspirin administration. The authors have thus confirmed the psychological findings by an independent and objective study of human brain waves.

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