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PRL-8-53: Enhanced Learning and Subsequent Retention in Humans as a Result of Low Oral Doses of New Psychotropic Agent

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Abstract. The effect of 3-(2-benzylmethylaminoethyl) benzoic acid methyl ester hydrochloride (PRL-8-53) on learning and on retention of verbal information in human subjects was investigated. Using the serial anticipation method under double-blind conditions it was found that PRL-8-53 causes slight improvement of acquisition. Retention of verbal information was found improved to a statistically significant degree (most P values better than 0.01, some better than 0.001). No significant changes were found for either visual reaction time or motor control after drug when compared with placebo values.

Key words: Learning – Memory – Benzoic acid derivatives – Phenethylamines

Early findings related to drug-induced facilitation of learning and memory have been reviewed by McGaugh and Petrinovich (1965). New studies and new compounds are being reported continuously (Wolthuis, 1971). The role of RNA and protein synthesis in learning and memory has been reviewed by Glassman (1969) and, more recently, the effect of RNA precursors has attracted attention (Ott and Mathies, 1973). Compounds affecting the adrenergic and cholinergic systems have been studied in detail (Krivaneck and McGaugh, 1969; Merlo and Izquierdo, 1971; Robichaud et al., 1973; Deutsch and Lutzky, 1967; Rech, 1968; Erickson, 1971). In summary, it appears that intellectual performance and, more particularly, retention of information can be positively affected along two major pathways: (1) Facilitation of RNA synthesis during and/or shortly after the learning experience will improve retention of the animal; (2) The action of certain analeptic stimulants and of some 'adrenergic' CNS agonists will enhance, while the effect of other CNS active agonists will diminish, intellectual performance. It seems to be necessary for optimal functioning to have an optimal balance of activity of all agonists and conductive systems concerned. This balance may vary from species to species. While in the rat a cholinergic increase may be required for optimal performance (Rech, 1968), the opposite may be true for man (Boshes, 1970). A reduction of serotonergic activity seems to benefit the experimental animal (Tenen, 1967; Schlesinger et al., 1968). An increase in dopaminergic activity appears to be beneficial in man, or at least in the Parkinson patient (Boshes, 1970).

This paper reports the results obtained with a novel monoamine, identified as 3-(2-benzylmethylamino ethyl) benzoic acid methyl ester hydrochloride (PRL-8-53). The chemistry of this and related compounds is described in detail elsewhere (Hoffmann-La Roche, 1973; Hansl, 1974a). The compound is a benzoic acid derivative containing within its structure the phenethylamine skeleton. It also meets structural criteria required for interaction with cholinergic receptors. PRL-8-53 has produced a cholinergic response in the experimental animal. The compound potentiates dopamine and causes partial inhibition of serotonin (Hansl, 1973, 1974b). A compound with this profile might be expected to cause a favorable shift of the balance of CNS transmitter systems, thereby improving intellectual functioning.

PRL-8-53 exhibits low toxicity with an oral LD_{50} of 860 mg/kg in mice. Blood pressure of the normotensive dog is not significantly affected by doses of up to 8 mg/kg. Higher doses cause a slight drop in pressure of brief duration (Hansl, 1974b). The compound depresses motor activity of the mouse and rat. For the mouse the ED_{50} for a 50% reduction is 160 mg/kg. PRL-8-53 does not produce stimulation or depression of avoidance rate in rats working on a continuous avoidance schedule. The compound, at 20 mg/kg, does not potentiate the stimulant effects

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of *d*-amphetamine in rats in the continuous avoidance test. No signs of amphetaminelike stereotypic behavior are seen after doses up to 200 mg/kg of PRL-8-53. Reserpine-induced catatonia and ptosis is reversed by PRL-8-53.

The main objective of this study was to determine the effects of PRL-8-53 on learning and retention of verbal information in man. The secondary objective was to determine its effects on both visual reaction time and motor control.

MATERIALS AND METHODS

All subjects received either a capsule containing magnesium trisilicate (placebo) or 5 mg of PRL-53 in a capsule mixed with magnesium trisilicate as filler.

A total of 47 volunteers recruited from the faculty and students at the university participated in the study. All were normal, healthy adults. All tests were done on a double-blind basis. The major testing device was a modification of the serial anticipation test used by R. G. Smith (1967). For this verbal test, a number of word lists were prepared, each consisting of 12 one-syllable, three- or four-letter English words. The lists were matched as to difficulty. A detailed description and discussion of the lists will be presented elsewhere. The recorded word lists were presented audibly to the subjects by the serial anticipation method. The words were heard at 3-s intervals with an 8-s intertrial interval, and each list of 12 words was repeated nine times for each individual session. The number of correct anticipations was recorded for each of eight trials. A complete 12 word list was used for an orientation and familiarization session, but no retention scores were recorded. To determine retention 24 h after and 4 days after every test, each subject was instructed to enter on a prepared form all the words, if possible, in their proper sequence, which were recalled from the last test. A total of 5 min was allowed for this task.

The number of words recalled (gross score) and the number of words recalled in their correct order (discrete score) were subsequently used as the measures of retention performance. Individual tests were given in small private offices with only the subject and the recorder present. Each subject took the capsule of either drug or placebo $2-2\frac{1}{2}$ h before the time. The random distribution of placebo and drug was arranged so that, with the exception of the familiarization trials, 50% of the subjects (for any given word list) would have taken placebo and 50% would have taken 5 mg of PRL-8-53. This procedure largely eliminated any effects on performance due to possible variation in the difficulty among word lists. All subjects participated in two conditioning trials, complete in every detail but for the use of recorded data. This was done to eliminate as much as possible apprehension and the occasionally encountered over-eagerness caused by the novelty of the situation.

A Lafayette visual reaction time apparatus and timer measured the visual reaction time. A series of six determinations was repeated twice with a short rest interval after the first six trials. The result of the first determination in each group was discarded and the average time for the remaining ten determinations was entered as the reaction time for that day.

To measure motor control, a standard hand steadiness test was used (steadiness tester: hole type 4605 C, Lafayette). Subjects inserted a stylus three times in a row into one of seven consecutively smaller holes for a period of 5 s each. The number of contacts between stylus and frame was automatically recorded. The total for the 21 individual attempts was entered as the hand steadiness value for the day.

RESULTS

Tables 1–4 summarize the results. For statistical reasons, only data for those subjects who participated in evenly paired tests (i.e., one drug and one placebo test had to be available) were used. In conformity with other clinical studies, which will be reported elsewhere, subgroups of the total population are considered. Subjects with a discrete recall score of 6 words or less (50% achievement level or less) are treated in Table 2. Over 60% of the total population fall into this subgroup. A second group of subjects who recalled 8 words or more (discrete) after 24 h is treated as a

Table 1. Population 1A (total population)

	Score	*n*	Mean	*t*	*P*	Change (%)
Acquisition						
Placebo	3070	52	59			
8-53	3296	52	63.4	2.2221	< 0.02	+ 7.46
24-h retention						
Placebo						
Discrete	222	43	5.16			
Gross	242	43	5.63			
8-53						
Discrete	317	43	7.37	5.8789	< 0.001	+ 42.7
Gross	339	43	7.78	5.4435	< 0.001	+ 32.5
1-week retention						
Placebo						
Discrete	139	35	3.97			
Gross	145	35	4.14			
8-53						
Discrete	202	35	5.77	3.9630	< 0.001	+ 45.2
Gross	212	35	6.06	4.0901	< 0.001	+ 46.2

Table 2. Population 1B (subjects with 24-h recall of 6 words or less on placebo only)

	Score	*n*	Mean	*t*	*p*	Change (%)
Acquisition						
Placebo	1650	31	53.23			
8-53	1959	31	63.19	4.7386	< 0.001	+ 18.7
24-h retention						
Placebo						
Discrete	90	28	3.25			
Gross	106	28	3.79			
8-53						
Discrete	173	28	6.21	6.2707	< 0.001	+ 91
Gross	190	28	6.75	5.3856	< 0.001	+ 87.5
1-week retention						
Placebo						
Discrete	57	25	2.28			
Gross	62	25	2.48			
8-53						
Discrete	117	25	4.68	4.4069	< 0.001	+ 105
Gross	124	25	4.96	4.3145	< 0.001	+ 100

Table 3. Population 1C (subjects with 24-h recall of 8 words or more on placebo only)

	Score	*n*	Mean	*t*	*P*	Change (%)
Acquisition						
Placebo	1160	16	72.56			
8-53	1110	16	69.38	1.2851	> 0.05	− 4
24-h retention						
Placebo						
Discrete	117	13	9.0			
Gross	121	13	9.3076			
8-53						
Discrete	129	13	9.923	1.8972	> 0.05	+ 10
Gross	138	13	10.6153	3.0951	< 0.02	+ 14
1-week retention						
Placebo						
Discrete	75	9	8.33			
Gross	76	9	8.44			
8-53						
Discrete	81	9	9.0	1.0689	> 0.05	+ 7.9
Gross	83	9	9.22	1.2571	> 0.05	+ 9.2

separate unit. (There were no subjects with a recall of seven words and only one with eight.)

Statistical analysis for total population indicates only borderline significance for drug effect on acquisition, but 24-h and 1-week retention improvement is highly significant with P values better than 0.001. (Student's t-test, one-tailed.)

Pronounced effects are found in the subgroup of subjects with a 24-h recall on placebo of 6 words or less. Here even acquisition is improved by 18%. The retention average for the subjects on drug is increased by 87−105%. The extent of improvement implies potential utility and underlines the statistical significance of P less than 0.001.

Only the subgroup of high achievers fails to show significant improvement across the board. It must be kept in mind, however, that it is near impossible to obtain significant improvement figures when the control performance is near perfect to begin with. To evaluate meaningfully the effect of PRL-8-53 for this

Table 4. Population 1D (subjects over 30 years of age)

	Score	*n*	Mean	*t*	*P*	Change (%)
Acquisition						
Placebo	692	17	40.71			
8-53	909	17	53.47	3.7951	< 0.001	+ 31.4
24-h retention						
Placebo						
Discrete	41	17	2.42			
Gross	48	17	2.82			
8-53						
Discrete	91	17	5.35	4.0572	< 0.001	+ 122
Gross	100	17	5.88	3.9280	< 0.001	+ 108
1-week retention						
Placebo						
Discrete	25	12	2.09			
Gross	25	12	2.09			
8-53						
Discrete	59	12	4.92	3.2284	< 0.01	+ 136
Gross	63	12	5.25	3.5063	< 0.01	+ 152

subgroup, a more difficult test instrument will have to be used.

Subjects over 30 years of age responded very well as a group. Acquisition is improved by a full 31%. Retention scores show a 108–152% improvement after drug. It would seem that our intellectual faculties, as they begin to deteriorate with age, are still amenable to at least a partial restoration through drug action.

Two other areas of human performance were investigated. Visual reaction time showed a very minor improvement following drug, but it was not statistically significance. Motor control was slightly superior in the drug group, but this effect is also of no statistical significance. This finding seems important because of the absence of a negative response, the type of adverse effect that might be expected for a phenethylamine-related compound.

Side Effects. Inquiry was made of all subjects regarding potential side effects. Not a single instance of an adverse reaction was reported.

DISCUSSION

The title compound, PRL-8-53, has been reported previously to improve acquisition and retention in the experimental animal (Hansl, 1974a, 1975). These positive effects on conditioned avoidance responses in rats lend support to the original hypothesis that links certain pharmacological activities to enhancement of intellectual functions. The present clinical study lends further credence to the concepts of optimal balance of conductive systems in the CNS, and to the projection that the existing balance in a given individual can be successfully modulated. The fact that a large proportion of the population responded favorably to drug treatment may indicate that for the majority of people a similar pattern of imbalance develops. This correlation might be even more pronounced for people advancing with age, if the test results of this study with the population subgroup over 30 years of age can be taken as an indicator.

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NOTE ADDED IN PROOF

Following completion of this study it was found that the particular lot of PRL-8-53 that had been used contained a small amount of the desmethyl compound as admixture, which may also have contributed to the observed effects. However, since this compound is considered a metabolite of the parent compound (8-53), its presence should not detract from the findings.